Synthesis and evaluation of 6-aza-2'-deoxyuridine monophosphate analogues as inhibitors of thymidylate synthases in *Mycobacterium tuberculosis*

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Abstract

A series of 5-substituted 6-aza-2'-deoxyuridine-5'-monophosphate analogues 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 the carbon at position 6 of the natural substrate dUMP by nitrogen 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 12-carbon propargylamide side chain in derivative **3** retained the weak level of mycobacterial ThyX inhibition (40 % inhibition at 50 μM). None of the synthesized derivatives displays any significant inhibitory activity against mycobacterial ThyA.

Keywords

Flavin-dependent thymidylate synthase, 6-azauracil nucleotides, tuberculosis

Introduction

The emergence of Multi-drug resistant (MDR) and more recently, extremely drug resistant (XDR) strains of *Mycobacterium tuberculosis* has complicated the successful implementation of 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-programme) with first line drugs. The standard regimen comprises of a 2 month initiation period with isoniazid, rifampin, pyrazinamide and ethambutol followed by a 4 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] (eg. 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} of 1.26 μM which was able to restore IFN-γ induced apoptosis in Raw264.7 macrophages expressing mPTPB (*Fig. 1*).

Figure 1

Bacterial thymidylate synthase 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 thymidylate synthase 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 thymidylate synthase inhibitors is very challenging. Recent genomic analysis has demonstrated 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'-monophasphate (dUMP) to thymidine-5'-monophosphate which constitutes an essential step in nucleotide metabolism. While ThyA uses N^5,N^{10}-methylene tetrahydrofolic 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 fulfil 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 proton at C-(5) of dUMP which generates an *exo*-methylene bond at C-(5). An intramolecular 1,3-hydride shift, finally, furnishes dTMP (*Scheme 1*).

Scheme 1

ThyA and ThyX show neither structural nor sequence 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 data, mycobacterial ThyX was selected as a promising target for the discovery of novel tuberculosis medication. Recently, C-(5)-aryl and alkynyl dUMP derivatives have been developed in our laboratory as selective ThyX-inhibitors (Figure 1) [22]. In order to further study the structure-activity relationship (SAR) of dUMP analogues as mycobacterial ThyX inhibitors, we envisioned to prepare 6-aza dUMP analogues in which C-(6) of the uracil moiety is replaced by a nitrogen atom. This would preclude the nucleophilic attack of a hydride from $FADH_2$. In this paper, the synthesis and biological evaluation of 6-aza dUMP analogues, whose structures are depicted in *Figure 2*, is described.

Figure 2

Results and discussion

Chemistry. The synthesis of 6-aza dUMP derivatives **1a-d** and **2-4** has been carried out via Vorbrüggen-type glycosylation [23] of suitable 5-substituted 6-azauracils with Hoffer's chlorosugar **7** [24]. Chlorosugar **7** was prepared in three steps from 2-deoxy-*D*-ribose **5** (*Scheme 2*). Upon treatment of intermediate **6** with saturated HCl in acetic acid, diastereomerically pure compound **7** precipitated out after a few minutes.

Scheme 2

The synthesis of 6-azathymine **10b** and 5-i-Pr-6-azauracil **10c**, shown 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-d were treated with thiosemicarbazide in EtOH followed by ring closure under aqueous alkaline conditions furnishing 2-thio-6-azauracil derivatives **9b-d** [25]. Conversion of **9b-d** to the desired 6-azauracil derivatives **10b-d** was performed in alkaline H_2O_2 at room temperature [25]. 5-Iodo-6-azauracil **10e** was prepared according to a literature procedure by iodination of commercially available 6-azauracil, albeit in low yield [26].

Scheme 3

Coupling reactions between 6-azauracil moieties **10a-e** and the chlorosugar **7** were performed in the presence of CuI as catalyst according to the method by *Freskos* (*Scheme 4*) [27]. Yields were usually good, however, in contrast to the literature report [27], only a modest *β*/*α*-selectivity (usually around 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 eq) led to a slight improvement of that ratio to around 70 % but can generally be omitted. The nucleosides **12a-d** were subsequently subjected to alkaline deprotection of the toluoyl groups [28], followed by phosphorylation of the primary hydroxyl groups [29], yielding the nucleoside monophosphate derivatives **1a-d**.

Scheme 4

Toluoyl protected 5-iodo-6-aza-2'-deoxy-uridine derivative **12e** served as a key intermediate for the preparation of 5-fluorophenyl-6-aza derivative **2,** as well as the 5-acetylenic-6-aza derivative **3** (*Scheme 5*). Coupling of compound **12e** with 4-fluorophenylboronic acid under Suzuki-Liebeskind reaction conditions [30]

led to the corresponding toluoyl protected nucleoside derivative. Alkaline deprotection, followed by phosphorylation of the primary hydroxyl group of derivative **14** yielded the 5-(4-flurophenyl)-nucleotide derivative **2**. Sonogashira coupling [31] of **12e** with *N*-(prop-2-ynyl)octanamide [22] under standard reaction conditions using $Pd(PPh_3)_4$ 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 this 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**.

Scheme 5

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

These intriguing findings indicate the presence of two enolizable methylene protons adjacent to a carbonyl 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 DMSO-d_6 as solvent, enolization was impossible, and two "normal" CH_2 peaks were obtained instead of the CD_2-peaks (*Fig. 3*).

Figure 3

Furthermore, the protons of the two methylene groups 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 two methylene protons *β* to the carbonyl group. The correct structure of analogue **4** was further confirmed by 2D-COSY-NMR spectroscopy (*Fig. 4*). In DMSO-*d*$_6$ (in contrast to the spectrum in D_2O), the ^{1}H-NMR spectrum showed the presence of two additional methylene groups at δ = 3.65 and 4.0, respectively. In the 2D-COSY spectrum, no cross-peak between these two methylene groups was observed. It rules out the correct structure to be regioisomer **17**.

Figure 4

The correct connection of the nucleobase to the 2'-deoxyribose moiety was determined by 2D-HMBC-NMR spectroscopy using compound **1c** as representative example. *Figure 5* shows the proton H-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**.

Figure 5

Biological Evaluation

Compounds have been evaluated for their inhibitory activity against mycobacterial ThyX and ThyA. Cloning of the ThyX and ThyA gene, protein expression and purification was done as published previously [22]. Similarly, the biochemical assays have been performed as reported before [22,32]. Compounds were evaluated at a concentration of 50 μM in the ThyX, as well as

in the ThyA, assay. In both assays, 5-FdUMP was included as reference compound and positive control.

The co-crystal structure [33] of the ternary complex ThyX-FAD-5-BrdUMP revealed that the oxygen atoms of the 5'-monophosphate moiety of 5-Br-dUMP are engaged in crucial interactions with residues in the active site of the ThyX enzyme. Furthermore, the pyrimidine ring of 5-BrdUMP and the isoalloxazine ring of FAD are close enough to interact *via* hydrogen bonds and π-stacking. Based on this information from X-ray crystallography studies, the structure-activity relationship (SAR) study at position 5 of the natural dUMP substrate has been studied already quite extensively. The presence of small substituents (fluorine, bromine) led to potent ThyX inhibitory activity. Furthermore, it has been demonstrated by our group that introduction of sterically demanding substituents such as a 4-fluoro-phenyl moiety (compound **VI**) and a long-chain long propargylamide side chain (compound **V**), yielded nucleotide derivatives with potent and selective mycobacterial ThyX inhibitory activity, displaying IC_{50} values of 10 and 0.91 μM, respectively [22]. The fact that quite some structural variety is tolerated at position 5 of the natural uracil base prompted us to prepare a series of 6-aza dUMP analogues, in which the 5-substituent was varied in a systematic way. Because of the above mentioned crucial interactions of the phosphate group, all synthesized derivatives in this paper 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 @ 50 μM, Table 1). In order to increase activity, small modifications were introduced at C-5 of the 6-azauracil base moiety, starting with a methyl

group in analogue **1b.** This resulted in a complete loss of inhibitory activity. Similarly, increasing the size of the substituent to an *i*-propyl group (compound **1c)** or an *n*-octyl chain (compound **1d)** led to complete loss of ThyX-inhibitory activity.

As within the dUMP series, a 4-fluorophenyl group and a long propargylamide 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 propargylamide side chain). As can be derived from Table 1, compound **2** lacks completely any inhibitory activity, whereas derivative **3** displays only low levels of ThyX inhibitory activity (40 % inhibition at 50 μM). Both analogues are totally devoid of ThyA-inhibitory activity. These data highlight the fact that replacement of the carbon at position 6 by a nitrogen is detrimental for ThyX inhibitory activity.

Table 1

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. Herein, the synthesis of novel 6-aza dUMP analogues with structural variation at C-5 of the base moiety is

described. These compound 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 propargylamide side chain displayed weak ThyX inhibition (30-40% inhibition @ 50 μM) and completely lacked any activity against ThyA. Further input from structural biology, X-ray crystallographic studies and modelling are required to provide a rationale for these findings.

Acknowledgement

The authors are grateful to Mr. Luc Baudemprez for running 500 and 600 MHz NMR-spectra and to Mrs. Chantal Biernaux for editorial help. This work was partly funded by a collaborative project grant (NATT-project) from the European Commission. *M. K.* is deeply indebted to the IWT (*Agentschap voor Innovatie door Wetenschap en Technologie*) for providing a PhD-scholarship.

Experimental Section

General

For all reactions, analytical grade solvents were used. Dry MeOH was obtained by distillation over CaH_2. Dry $CHCl_3$, THF, diethyl ether, and DMF were purchased from commercial suppliers. All moisture-sensitive reactions were carried out in oven-dried glass-ware (120°). ^{1}H and ^{13}C-NMR spectra were recorded with a *Bruker Advance 300* (^{1}H-NMR: 300 MHz, ^{13}C-NMR: 75 MHz, ^{31}P-NMR: 121 MHz) or 500 MHz (^{1}H-NMR: 500 MHz, ^{13}C-NMR: 125 MHz) spectrometer using tetramethylsilane as internal standard for ^{1}H-NMR spectra and (D_6)-DMSO (39.52 ppm) or $CDCl_3$ (77.16 ppm) for ^{13}C-NMR spectra. Abbreviations used are: *s* = singlet, *d* = doublet, *t* = triplet, *q* = quartet, *m* = multiplet, br. *s* = broad singlet. Chemical shifts are expressed in parts per million

(ppm). Coupling constants are expressed in Hz. Mass spectra are obtained with a *Finnigan LCQ advantage Max* (ion trap) mass spectrometer from *Thermo Finnigan*, San Jose, CA, USA. Exact mass measurements were performed on a quadrupole time-of-flight mass spectrometer (*Q-tof-2*, *Micromass*, Manchester, UK) equipped with a standard electrospray-ionization (ESI) interface. Samples were infused in i-PrOH/H_2O (1:1) at 3 µl/min. Precoated aluminum sheets (*Fluka* Silica gel/TLC-cards, 254 nm) were used for TLC. Column chromatography was performed on ICN silica gel 63-200, 60 Å. All final compounds possess a purity of at least 95% as determined by analytical RP-HPLC analysis on an *XBridge* column (C-18, 5 µm, 4.6 x 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. Preparative HPLC purification was carried out on the same instrument using a preparative *XBridge* column (C-18, 5 µm, 19 x 150 mm) from Waters, Milford, Massachusetts, USA.

2-Deoxy-3,5-di-O-p-toluoyl-D-erythro-pentofuranosyl chloride (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 subsequently added. The reaction mixture was stirred at r.t. for 25 minutes, and then quenched through addition of solid $NaHCO_3$ (1.6 g). The resulting suspension was stirred for about 5 min, then filtered off, washed with MeOH, and evaporated *in vacuo*. The residue was coevaporated twice with dry pyridine and subsequently dissolved in dry pyridine (24 ml) under an Ar atmosphere and cooled to 0°. *p*-Toluoyl chloride (8.8 ml, 65.8 mmol) was added drop- to portion wise, the ice bath was removed, and the reaction 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 x 40 ml). The org. layer was washed

with satd $NaHCO_3$ (1 x 100 ml), 2 N HCl (2 x 100 ml) and H_2O (1 x 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 saturated soln. of HCl in AcOH (25 ml) at r.t. Towards the end of the addition, the desired product **7** (7.07 g, 61% over 3 steps) readily precipitated thereby forming a thick milky suspension which was rapidly filtered off, washed with dry ether, and dried *in vacuo*. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.99 (*d*, *J* = 8.2, 2H, ArH); 7.89 (*d*, *J* = 8.2, 2H, ArH); 7.25 (*m*, 4H, ArH); 6.47 (*d*, *J* = 5.1, 1H, H-1); 5.56 (*m*, 1H, H-3); 4.86 (*m*, 1H, H-4); 4.71-4.57 (*ddd*, *J* = 42.6, 12.1, 3.7, 2H, H-5); 2.91-2.82 (*m*, 1H, H-2); 2.74 (*d*, *J* = 9.0, 1H, H-2); 2.42 (*s*, 3H, CH_3); 2.41 (*s*, 3H, CH_3). ^{13}C-NMR (125 MHz, $CDCl_3$): 166.55, 166.21, 144.44, 144.21, 130.06 (2C), 129.82 (2C), 129.39 (2C), 129.36 (2C), 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).

6-Methyl-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-5(2H)-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 minutes. 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 minutes. The resulting clear soln. was allowed to cool to r.t. and acidified to pH = 4 with AcOH at 0°. The aqueous layer was extracted with AcOEt (3 x 40 ml), the org. layer was dried ($MgSO_4$) and evaporated, which afforded pure compound **9b** (1.53 g, 86%) as a white solid. ^{1}H-NMR (300 MHz, $CDCl_3$:MeOD = 6:1): 2.25 (*s*, 3H, CH_3). ^{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).

6-i-Propyl-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-5(2H)-one (9c).

To a soln. of ethyl dimethyl pyruvate **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 minutes. 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 minutes. The resulting clear soln. was allowed to cool to r.t. and acidified to pH = 4 with AcOH at 0°. The aqueous layer was extracted with AcOEt (3 x 40 ml), the org. layer was dried ($MgSO_4$) and evaporated, which afforded pure compound **9c** (1.86 g, 95%) as a white solid. ^{1}H-NMR (300 MHz, $CDCl_3$:MeOD = 7:1): 3.17 (*m*, 1H, CH i-Pr); 1.22 (*s*, 3H, CH_3); 1.19 (*s*, 3H, CH_3). ^{13}C-NMR (75 MHz, $CDCl_3$:MeOD = 7:1): 173.44, 155.10, 152.70, 28.66, 19.44 (2C). ESI-MS (neg.): 169.80 ($[M - H]^-$, $C_6H_8N_3OS^-$; calc. 170.04).

6-Octyl-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-5(2H)-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 an Ar atmosphere, and the soln. was cooled to -60°. *n*-Octylmagnesium bromide (2 M in Et_2O, 4.4 ml, 8.8 mmol) was added over 10 minutes, and the stirred reaction mixture was allowed to warm up to 0° over 2 ½ h. The reaction was quenched through addition of 2 N H_2SO_4 (7 ml), diluted with H_2O, and the phases were separated. The aqueous layer was extracted with Et_2O (1 x 15 ml), the org. phase was dried ($MgSO_4$) and evaporated. The resulting yellow oil **8d** (1.84 g, quantitative yield) 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 minutes, 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 minutes, allowed to cool to r.t., and acidified to pH = 4 with AcOH at 0 °C. The suspension was diluted with H_2O (25 ml), extracted with AcOEt (3 x 40 ml), and the org. layer was dried ($MgSO_4$) and evaporated. The residue was purified by silica gel column chromatography (petroleum ether/AcOEt = 4:1 containing 1% formic acid) to yield compound **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 (10b).

Compound **9b** (716 mg, 5 mmol) was dissolved in 1 M NaOH (15 ml), and the soln. was cooled to 0°. 35% aq H_2O_2 (1.9 ml, 22.27 mmol) was added dropwise, whereupon the colour of the reaction mixture changed from colourless to yellow and finally back to colourless towards the end of the H_2O_2-addition. The reaction mixture was stirred at r.t. for 30 minutes and subsequently acidified with conc. HCl at 0°. The aqueous layer was extracted with AcOEt (6 x 30 ml), the combined organic 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*, 3H, CH_3). ^{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-i-Propyl-6-azauracil (10c).

Compound **9c** (1.20 g, 7 mmol) was dissolved in 1 M NaOH (21 ml), and the soln. was cooled to 0°. 35% aq H_2O_2 (2.65 ml, 31.06 mmol) was added dropwise, whereupon the colour of the reaction mixture changed from colourless

to yellow and finally back to colourless towards the end of the H_2O_2-addition. The reaction mixture was stirred at r.t. for 30 minutes and subsequently acidified with conc. HCl at 0°. The aqueous layer was extracted with AcOEt (6 x 30 ml), the combined organic layer was dried ($MgSO_4$) and evaporated which afforded pure **10c** (830 mg, 76%) as a white solid. ^{1}H-NMR (300 MHz, $CDCl_3$:MeOD = 6:1): 3.15 (*m*, 1H, CH i-Pr); 1.21 (*s*, 3H, CH_3); 1.18 (*s*, 3H, CH_3). ^{13}C-NMR (75 MHz, $CDCl_3$:MeOD = 6:1): 156.81, 150.82, 150.22, 28.55, 19.62 (2C). ESI-MS (neg.): 153.90 ([M – H]$^-$, $C_6H_8N_3O_2^-$; calc. 154.06).

5-Octyl-6-azauracil (10d).

Crude **9d** (1.02 g, 4.23 mmol) was suspended in 1 M NaOH (13 ml), and 35% aq H_2O_2 (1.6 ml, 18.75 mmol) was added dropwise at r.t. The reaction mixture was stirred at r.t. for 30 min, then acidified with conc. HCl, and diluted with H_2O (30 ml). The aqueous layer was extracted with AcOEt (3 x 40 ml), the organic layer was dried ($MgSO_4$) and evaporated *in vacuo*.The residue was slurried up in petroleum ether, filtered off, and washed with petroleum ether to afford compound **10d** (392 mg, 41%) as a white solid. ^{1}H-NMR (300 MHz, $CDCl_3$:MeOD = 6:1): 2.57 (*t*, J = 7.7, 2H, α-CH_2); 1.61 (*m*, 2H, β-CH_2); 1.27 (*m*, 10H, 5 x CH_2); 0.87 (*t*, J = 6.8, 3H, CH_3). ^{13}C-NMR (75 MHz, $CDCl_3$:MeOD = 6:1) δ 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 (10e).

To a soln. of 6-azauracil **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 decolourized with 5% $NaHSO_3/Na_2S_2O_5$-soln. (60 ml) at 0° and subsequently extracted with AcOEt (3 x 150 ml). The organic layer was washed with a soln. containing 150 ml H_2O and 10 ml 5% $NaHSO_3/Na_2S_2O_5$, dried ($MgSO_4$) and evaporated. The residue was purified by silica gel column chromatography (2% EtOH in CH_2Cl_2 containing 1% formic acid), which afforded compound **10e** (1.21 g, 17%) as an off-white solid. ^{1}H-NMR (300 MHz, (D_6)-DMSO): 12.60 (br. *s*, 1H, NH); 12.24 (br. *s*, 1H, 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 (12a).

A suspension of 6-azauracil **10a** (372 mg, 3.3 mmol) and $(NH_4)_2SO_4$ (39 mg, 0.3 mmol) in HMDS (20 ml) was refluxed overnight under an Ar atmosphere. 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 chlorosugar **7** (1.17 g, 3 mmol), CuI (573 mg, 3 mmol), and dry $CHCl_3$ (60 ml), and the reaction mixture was stirred at r.t. for 2 h. The reaction mixture was quenched through addition of satd $NaHCO_3$ (5 ml), 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 satd $NaHCO_3$ (1 x 60 ml) and brine (1 x 60 ml), dried ($MgSO_4$) and evaporated. The residue was purified by silica gel column chromatography (1% EtOH in CH_2Cl_2) to yield compound **12a** as an anomeric mixture. Recrystallization from hot EtOH afforded pure *β*-anomer **12a** (810 mg, 58%) as a white solid. ^{1}H-NMR (300 MHz, $CDCl_3$): 9.36 (br. *s*, 1H, NH); 7.95 (*m*, 4H, ArH); 7.25 (*m*, 4H, ArH); 6.69 (*t*, *J* = 6.4, 1H, H-1'); 5.72 (*m*, 1H, H-3'); 4.66 (*m*, 1H, H-4'); 4.56-4.40 (*m*, 2H, H-5'); 3.04-2.93 (*m*, 1H, H-2'); 2.55-2.35 (*m*, 1H, H-2'); 2.43 (*s*, 3H, CH_3); 2.41

(*s*, 3H, CH_3). ^{13}C-NMR (75 MHz, $CDCl_3$): 166.36, 166.16, 155.58, 148.05, 144.51, 144.07, 136.19, 129.95 (2C), 129.91 (2C), 129.37 (2C), 129.25 (2C), 127.15, 126.67, 86.07, 82.72, 75.04, 64.09, 35.18, 21.85, 21.82. ESI-MS (pos.): 488.10 ($[M + Na]^+$, $C_{24}H_{23}N_3O_7Na^+$; calc. 488.14).

3',5'-Di-*O*-p-toluoyl-2'-deoxy-6-azathymidine (12b).

A suspension of 6-azathymine **10b** (210 mg, 1.65 mmol) and $(NH_4)_2SO_4$ (20 mg, 0.153 mmol) in HMDS (15 ml) was refluxed overnight under an Ar atmosphere. The clear soln. obtained was allowed to cool to r.t., and the solvent was evaporated under exclusion of air and moisture. Chlorosugar **7** (585 mg, 1.5 mmol) was added, and the mixture was dissolved in dry $CHCl_3$ (30 ml) under an Ar atmosphere. TMSCl (192 µl, 1.5 mmol) and CuI (287 mg, 1.5 mmol) were then added, and the reaction mixture was stirred at r.t. for 3 h. The reaction mixture was quenched through addition of satd $NaHCO_3$ (3 ml), 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 satd $NaHCO_3$ (1 x 40 ml) and brine (1 x 40 ml), dried ($MgSO_4$) and evaporated. The residue was purified by silica gel column chromatography (3-5% acetone in CH_2Cl_2) to yield compound **12b** as an anomeric mixture. Recrystallization from hot EtOH afforded pure *β*-anomer **12b** (525 mg, 73%) as a white solid. ^{1}H-NMR (300 MHz, (D_6)-DMSO): 12.15 (br. *s*, 1H, NH); 7.88 (*m*, 4H, ArH); 7.33 (*m*, 4H, ArH); 6.49 (*t*, *J* = 6.0, 1H, H-1'); 5.63 (*m*, 1H, H-3'); 4.58-4.37 (*m*, 3H, H-4' and H-5'); 2.94-2.77 (*m*, 1H, H-2'); 2.60-2.20 (*m*, 1H, H-2'); 2.40 (*s*, 3H, CH_3); 2.38 (*s*, 3H, CH_3); 2.03 (*s*, 3H, CH_3). ^{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→8C], 126.69, 126.49, 85.00, 81.11, 74.79,

64.13, 34.38, 21.15 (2C), 16.11. ESI-MS (pos.): 502.10 ([*M* + Na]$^+$, $C_{25}H_{25}N_3O_7Na^+$; calc. 502.16).

5-i-Propyl-3',5'-di-*O*-p-toluoyl-2'-deoxy-6-azauridine (12c).

A suspension of 5-*i*-propyl-6-azauracil **10c** (341 mg, 2.2 mmol) and $(NH_4)_2SO_4$ (25 mg, 0.191 mmol) in HMDS (20 ml) was refluxed overnight under an Ar atmosphere. The clear soln. obtained was allowed to cool to r.t., and the solvent was evaporated under exclusion of air and moisture. Chlorosugar **7** (780 mg, 2 mmol) was added, and the mixture was dissolved in dry $CHCl_3$ (40 ml) under an Ar atmosphere. TMSCl (256 µl, 2 mmol) and CuI (383 mg, 2 mmol) were then added, and the reaction mixture was stirred at r.t. for 3 h. The reaction mixture was quenched through addition of satd $NaHCO_3$ (3 ml), 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 satd $NaHCO_3$ (1 x 50 ml) and brine (1 x 50 ml), dried ($MgSO_4$) and evaporated. The residue was purified by silica gel column chromatography (2% acetone in CH_2Cl_2) to yield pure *β*-anomer **12c** (434 mg, 43%) as a white solid. ^{1}H-NMR (300 MHz, (D_6)-DMSO): 12.15 (br. *s*, 1H, NH); 7.86 (*m*, 4H, ArH); 7.30 (*m*, 4H, ArH); 6.52 (*dd*, *J* = 7.0, 4.7, 1H, H-1'); 5.70 (*m*, 1H, H-3'); 4.56-4.35 (*m*, 3H, H-4' and H-5'); 3.09-2.98 (*m*, 1H, CH i-Pr); 2.91-2.79 (*m*, 1H, H-2'); 2.60-2.20 (*m*, 1H, H-2'); 2.39 (*s*, 3H, CH_3); 2.35 (*s*, 3H, CH_3); 1.16 (*d*, *J* = 6.9, 3H, CH_3); 1.12 (*d*, *J* = 6.9, 3H, CH_3). ^{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→8C], 126.61, 126.49, 85.21, 80.98, 74.87, 64.57, 34.51, 28.25, 21.19, 21.15, 19.75 (2C). ESI-MS (pos.): 530.0 ([*M* + Na]$^+$, $C_{27}H_{29}N_3O_7Na^+$; calc. 530.19).

5-Octyl-3',5'-di-*O*-p-toluoyl-2'-deoxy-6-azauridine (12d).

A suspension of 5-octyl-6-azauracil **10d** (248 mg, 1.1 mmol) and $(NH_4)_2SO_4$ (13 mg, 0.1 mmol) in HMDS (10 ml) was refluxed overnight under an Ar atmosphere. 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 chlorosugar **7** (389 g, 1 mmol), CuI (191 mg, 1 mmol), and dry $CHCl_3$ (20 ml), and the reaction mixture was stirred at r.t. for 4 h. The reaction mixture was quenched through addition of satd $NaHCO_3$ (5 ml), 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 satd $NaHCO_3$ (1 x 40 ml), and the aqueous layer was extracted with AcOEt (1 x 40 ml). The combined organic layer was dried ($MgSO_4$) and evaporated. Upon purification by silica gel column chromatography (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, $CDCl_3$, only *β*-isomer given): 8.63 (br. *s*, 1H, NH); 7.93 (*m*, 4H, ArH); 7.23 (*m*, 4H, ArH); 6.69 (*t*, *J* = 6.4, 1H, H-1'); 5.70 (*m*, 1H, H-3'); 4.53 (*m*, 3H, H-4' and H-5'); 3.06-2.94 (*m*, 1H, H-2'); 2.66-2.45 (*m*, 3H, H-2' and *α*-CH_2); 2.43 (*s*, 3H, CH_3); 2.40 (*s*, 3H, CH_3); 1.60 (*m*, 2H, *β*-CH_2); 1.26 (*m*, 10H, 5 x CH_2); 0.86 (*t*, *J* = 6.7, 3H, CH_3). ^{13}C-NMR (75 MHz, $CDCl_3$, only *β*-isomer given): 166.33, 166.10, 155.60, 148.39, 148.04, 144.45, 143.99, [129.94, 129.36, 129.24→8C], 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 (2C), 14.23. ESI-MS (pos.): 600.10 ([*M* + Na]$^+$, $C_{32}H_{39}N_3O_7Na^+$; calc. 600.29).

5-Iodo-3',5'-di-*O*-p-toluoyl-2'-deoxy-6-azauridine (12e).

A suspension of 5-iodo-6-azauracil **10e** (789 mg, 3.3 mmol) and $(NH_4)_2SO_4$ (39 mg, 0.3 mmol) in HMDS (30 ml) was refluxed overnight under an Ar

atmosphere. 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 chlorosugar **7** (1.17 g, 3 mmol), CuI (573 mg, 3 mmol), and the mixture was suspended in dry $CHCl_3$ (60 ml). TMSCl (383 µl, 3 mmol) was added in one portion, and the reaction mixture was stirred at r.t. for 3 h. The reaction mixture was quenched through addition of satd $NaHCO_3$ (5 ml), 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 satd $NaHCO_3$ (1 x 80 ml) and brine (1 x 80 ml), dried ($MgSO_4$) and evaporated. The residue was purified by silica gel column chromatography (3-5% acetone in CH_2Cl_2) to yield compound **12e** as an anomeric mixture. Recrystallization from hot EtOH afforded pure *β*-anomer **12e** (798 mg, 45%) as a white solid. ^{1}H-NMR (300 MHz, (D_6)-DMSO): 12.43 (br. *s*, 1H, NH); 7.91-7.84 (*m*, 4H, ArH); 7.36-7.30 (*m*, 4H, ArH); 6.44 (*dd*, *J* = 6.8, 5.4, 1H, H-1'); 5.58 (*m*, 1H, H-3'); 4.46 (*m*, 3H, H-4' and H-5'); 2.85-2.78 (*m*, 1H, H-2'); 2.56-2.49 (*m*, 1H, H-2'); 2.39 (*s*, 3H, CH_3); 2.37 (*s*, 3H, CH_3). ^{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→8C], 126.70, 126.54, 112.29, 85.66, 81.41, 74.53, 64.39, 34.92, 21.27 (2C). ESI-MS (pos.): 614.07 ([*M* + Na]$^+$, $C_{24}H_{22}IN_3O_7Na^+$; calc. 614.04).

2'-Deoxy-6-azauridine (13a).

To a suspension of compound **12a** (350 mg, 0.752 mmol) in dry MeOH (10 ml) was added NaOMe (30%-soln. in MeOH, 200 µl, 1.05 mmol), and the reaction 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 silica gel column chromatography (10% EtOH in CH_2Cl_2) to yield the desired compound

13a (113 mg, 66%) as an off-white solid. ^{1}H-NMR (300 MHz, $CDCl_3$:MeOD = 5:2): 7.44 (*s*, 1H, H-5); 6.51 (*t*, *J* = 6.0, 1H, H-1'); 4.48 (*m*, 1H, H-3'); 3.94 (*m*, 1H, H-4'); 3.75-3.60 (*m*, 2H, H-5'); 2.66-2.50 (*m*, 1H, H-2'); 2.40-2.20 (*m*, 1H, H-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]$^+$, $C_8H_{11}N_3O_5Na^+$; calc. 252.0591).

2'-deoxy-6-azathymidine (13b).

To a suspension of compound **12b** (400 mg, 0.834 mmol) in dry MeOH (14 ml) was added NaOMe (30%-soln. in MeOH, 266 µl, 1.4 mmol), and the reaction 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 silica gel column chromatography (7-10% EtOH in CH_2Cl_2) to yield the desired compound **13b** (188 mg, 92%) as an off-white hygroscopic solid. ^{1}H-NMR (300 MHz, (D_6)-DMSO): 12.08 (br. *s*, 1H, NH); 6.31 (*dd*, *J* = 7.0, 5.5, 1H, H-1'); 5.15 (br. *s*, 1H, OH); 4.60 (br. *s*, 1H, OH); 4.27 (*m*, 1H, H-3'); 3.70 (*m*, 1H, H-4'); 3.49-3.43 (*dd*, *J* = 11.5, 5.4, 1H, H-5'); 3.39-3.33 (*dd*, *J* = 11.6, 6.2, 1H, H-5'); 2.44-2.37 (*m*, 1H, H-2'); 2.08 (*s*, 3H, CH_3); 2.07-2.0 (*m*, 1H, H-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]$^+$, $C_9H_{13}N_3O_5Na^+$; calc. 266.0748).

5-i-Propyl-2'-deoxy-6-azauridine (13c).

To a suspension of compound **12c** (434 mg, 0.855 mmol) in dry MeOH (14 ml) was added NaOMe (30%-soln. in MeOH, 273 µl, 1.44 mmol), and the reaction 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 silica gel column chromatography (5-7% MeOH in CH_2Cl_2) to yield the desired compound **13c** (223 mg, 96%) as a white solid. ^{1}H-NMR (300 MHz, (D_6)-DMSO): 12.06 (br. *s*, 1H, NH); 6.35 (*dd*, *J* = 7.2, 4.8, 1H, H-1'); 5.17 (br. *s*, 1H, OH); 4.62 (br. *s*, 1H, OH); 4.32 (*m*, 1H, H-3'); 3.69 (*m*, 1H, H-4'); 3.54-3.44 (*m*, 1H, H-5'); 3.43-3.33 (*m*, 1H, H-5'); 3.13-2.97 (*m*, 1H, CH i-Pr); 2.48-2.37 (*m*, 1H, H-2'); 2.14-2.03 (*m*, 1H, H-2'); 1.13 (*d*, *J* = 6.8, 3H, CH_3); 1.12 (*d*, *J* = 6.8, 3H, CH_3). ^{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]$^+$, $C_{11}H_{17}N_3O_5Na^+$; calc. 294.1061).

5-Octyl-2'-deoxy-6-azauridine (13d).

To a suspension of compound **12d** (380 mg, 0.658 mmol) in dry MeOH (12 ml) was added NaOMe (30%-soln. in MeOH, 228 µl, 1.2 mmol), and the reaction 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 silica gel column chromatography (5% EtOH in CH_2Cl_2) to yield the desired compound **13d** (195 mg, 87%) as an off-white hygroscopic solid. ^{1}H-NMR (300 MHz, $CDCl_3$:MeOD = 5:2): 6.53 (*t*, *J* = 6.3, 1H, H-1'); 4.50 (*m*, 1H, H-3'); 3.96 (*m*, 1H, H-4'); 3.77-3.62 (*m*, 2H, H-5'); 2.73-2.50 (*m*, 3H, H-2' and α-CH_2); 2.32-2.19 (*m*, 1H, H-2'); 1.71-1.52 (*m*, 2H, β-CH_2); 1.28 (*m*, 10H, 5 x CH_2); 0.89 (*t*, *J* = 6.0, 3H, CH_3). ^{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 (2C), 26.10, 22.46, 13.80. HR-ESI-MS (pos.): 364.1863 ([*M* + Na]$^+$, $C_{16}H_{27}N_3O_5Na^+$; calc. 364.1843).

5-(4-fluorophenyl)-2'-deoxy-6-azauridine (14).

Compound **12e** (473 mg, 0.8 mmol), 4-fluorophenylboronic 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 reaction mixture was stirred at 50 °C for 24 h and then the solvent was evaporated *in vacuo*. The residue was taken up in AcOEt (50 ml) and washed with satd $NaHCO_3$ (50 ml) and brine (50 ml), dried ($MgSO_4$) and evaporated. The crude mixture was purified by silica gel column chromatography (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 NaOMe (30%-soln. in MeOH, 255 µl, 1.34 mmol), and the reaction 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 silica gel column chromatography (5% MeOH in CH_2Cl_2), early and late fractions were combined, evaporated and subjected to a 2nd column using the same eluent to yield the desired compound **14** (106 mg, 41% over two steps) as a white solid. ^{1}H-NMR (300 MHz, $CDCl_3$:MeOD = 6:2): 7.99 (*m*, 2H, ArH); 7.14 (*t*, *J* = 8.7, 2H, ArH); 6.61 (*t*, *J* = 6.0, 1H, H-1'); 4.58 (*m*, 1H, H-3'); 3.96 (*m*, 1H, H-4'); 3.72-3.60 (*ddd*, *J* = 26.2, 11.9, 4.6, 2H, H-5'); 2.76-2.63 (*m*, 1H, H-2'); 2.40-2.26 (*m*, 1H, H-2'). ^{13}C-NMR (75 MHz, $CDCl_3$:MeOD = 6:2): 163.89 (*d*, J_{CF} = 251.2, 1C), 156.06, 148.64, 141.83, 130.37 (*d*, J_{CF} = 8.5, 2C), 127.62 (*d*, J_{CF} = 3.2, 1C), 115.27 (*d*, J_{CF} = 21.7, 2C), 87.10, 85.92, 71.06, 62.38, 37.68. HR-ESI-MS (pos.): 346.0814 ($[M + Na]^+$, $C_{14}H_{14}FN_3O_5Na^+$; calc. 346.0810).

***N*-(3-(5-(2'-deoxy-6-azauridine))prop-2-ynyl)octanamide (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 anhydrous DMF (12 ml) under an Ar atmosphere, and NEt_3 (432 µl, 3.11 mmol) and *N*-(Prop-2-ynyl)octanamide [22] (846 mg, 4.66 mmol) were then added. After being stirred 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 x 120 ml) and H_2O (2 x 120 ml), dried ($MgSO_4$) and evaporated. The residue was purified by silica gel column chromatography (5% acetone in CH_2Cl_2 containg 1% formic acid), followed by a second column on silica gel using 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 NaOMe (30%-soln. in MeOH, 164 µl, 0.863 mmol), and the reaction 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 silica gel column chromatography (5-7% MeOH in CH_2Cl_2) affording two compounds: the desired derivative **15** (73 mg, 11%, 2 steps) and side product **16** (87 mg, 12%, 2 steps) as slightly yellow oils. ^{1}H-NMR (300 MHz, $CDCl_3$:MeOD = 6:2): 6.50 (*dd*, *J* = 6.8, 5.1, 1H, H-1'); 4.51 (*m*, 1H, H-3'); 4.25 (*s*, 2H, α-CH_2); 3.96 (*m*, 1H, H-4'); 3.79-3.70 (*dd*, *J* = 11.9, 4.2, 1H, H-5'); 3.70-3.60 (*dd*, *J* = 11.9, 4.2, 1H, H-5'); 2.65-2.51 (*m*, 1H, H-2'); 2.35-2.20 (*m*, 1H, H-2'); 2.23 (*t*, *J* = 7.7, 2H, C*H*$_2$CONH); 1.63 (*m*, 2H, C*H*$_2$CH$_2$CONH); 1.30 (*s*, 8H, 4 x CH_2); 0.88 (*t*, *J* = 6.7, 3H, CH_3). ^{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]$^+$, $C_{19}H_{28}N_4O_6Na^+$; calc. 431.1901).

***N*-(3-(5-(2'-deoxy-6-azauridine))-3-oxopropyl)octanamide (side product 16).**

^{1}H-NMR (300 MHz, $CDCl_3$:MeOD = 6:2): 6.49 (*dd*, *J* = 6.8, 4.9, 1H, H-1'); 4.48 (*m*, 1H, H-3'); 4.22 (*d*, *J* = 1.6, 2H, COC*H*$_2$NH); 3.92 (*m*, 1H, H-4'); 3.79-3.55 (*m*, 2H, H-5'); 2.66-2.52 (*m*, 1H, H-2'); 2.34-2.19 (*m*, 1H, H-2'); 2.27 (*t*, *J* = 7.6, 2H, NHCOC*H*$_2$); 1.63 (*m*, 2H, NHCOCH$_2$C*H*$_2$); 1.29 (br. *m*, 8H, 4 x CH_2); 0.88 (*t*, *J* = 6.7, 3H, CH_3). ^{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-d, 14, 15, and 16.

To a soln. of an unprotected 5-substituted nucleoside derivative (1 eq) and proton sponge (1.5-3 eq) in trimethyl phosphate was added $POCl_3$ (1.5-3 eq) in one portion at 0°, and the reaction mixture was stirred at 0° for 3 h. The reaction 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 silica gel column chromatography using a gradient of 2-propanol:water:NH_4OH. The phosphates isolated after lyophilization were further purified by preparative RP-HPLC (C-18, 5µM, 19 x 150 mm) using a gradient of acetonitrile and TEAB (30 mM), yielding analytically pure compounds. Next, the phosphates were subjected to ion exchange (Dowex 50 WX-8, Na^+) and lyophilized, yielding the corresponding disodium salts.

2'-Deoxy-6-azauridine-5'-monophosphate (1a).

This compound was prepared according to the general procedure described above. The following amounts of starting material and reagents were employed: 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 reaction mixture was stirred at 0° for 3 h and worked up as described above, including silica gel column chromatography (2-propanol:NH_4OH:H_2O = 75:15:10→70:20:5→65:25:5) and RP-HPLC (30 mM aq TEAB:CH_3CN = 99:1→98:2, 16 ml/min), yielding title compound **1a** (59 mg, 40%) as a white solid. ^{1}H-NMR (500 MHz, D_2O): 7.54 (*s*, 1H, H-5); 6.51 (*dd*, *J* = 6.9, 5.4, 1H, H-1'); 4.60 (*m*, 1H, H-3'); 4.09 (*m*, 1H, H-4'); 3.93-3.85 (*m*, 1H, H-5'); 3.85-3.78 (*m*, 1H, H-5'); 2.75-2.66 (*m*, 1H, H-2'); 2.33-2.24 (*m*, 1H, H-2'). ^{13}C-NMR (125 MHz, D_2O) δ 162.59, 152.96, 136.22, 85.51, 85.01 (*d*, J_{CP} = 8.0, 1C), 71.0, 64.17 (*d*, J_{CP} = 4.7, 1C), 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 (1b).

This compound was prepared according to the general procedure described above. The following amounts of starting material and reagents were employed: 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 reaction mixture was stirred at 0° for 3 h and worked up as described above, including silica gel column chromatography (2-propanol:NH_4OH:H_2O = 85:10:5→80:10:10→75:15:10) and RP-HPLC (30 mM aq TEAB:CH_3CN = 97:3→80:20, 16 ml/min), yielding title compound **1b** (91 mg, 53%) as a white solid. ^{1}H-NMR (500 MHz, D_2O): 6.51 (*dd*, *J* = 7.0, 5.8, 1H, H-1'); 4.62 (*m*, 1H, H-3'); 4.07 (*m*, 1H, H-4'); 3.94-3.88 (*m*, 1H, H-5'); 3.85-3.79 (*m*, 1H, H-5'); 2.77-2.69 (*m*, 1H, H-2'); 2.27-2.17 (*m*, 1H, H-2'); 2.19 (*s*, 3H, CH_3). ^{13}C-NMR

(125 MHz, D_2O): 167.67, 158.16, 145.11, 85.64, 85.18 (*d*, J_{CP} = 7.7, 1C), 71.68, 64.50 (*d*, J_{CP} = 4.6, 1C), 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-i-Propyl-2'-deoxy-6-azauridine-5'-monophosphate (1c).

This compound was prepared according to the general procedure described above. The following amounts of starting material and reagents were employed: 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 ½ ml of $PO(OMe)_3$. The reaction mixture was stirred at 0° for 3 h and worked up as described above, including silica gel column chromatography (2-propanol:NH_4OH:H_2O = 85:10:5→ 80:10:10→75:15:10) and RP-HPLC (30 mM aq TEAB:CH_3CN = 95:5→90:10→85:15, 16 ml/min), yielding title compound **1c** (27.1 mg, 17%) as a white solid. ^{1}H-NMR (500 MHz, D_2O): 6.48 (*dd*, *J* = 7.4, 4.1, 1H, H-1'); 4.60 (*m*, 1H, H-3'); 4.07 (*m*, 1H, H-4'); 3.97-3.91 (*m*, 1H, H-5'); 3.84-3.77 (*m*, 1H, H-5'); 3.09 (*m*, 1H, CH i-Pr); 2.74-2.68 (*m*, 1H, H-2'); 2.33-2.26 (*m*, 1H, H-2'); 1.18 (*d*, *J* = 6.9, 3H, CH_3); 1.17 (*d*, *J* = 6.0, 3H, CH_3). ^{13}C-NMR (125 MHz, D_2O): 158.18, 152.06, 150.35, 85.56, 85.30 (*d*, J_{CP} = 7.5, 1C), 71.45, 65.20 (*d*, J_{CP} = 4.5, 1C), 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 (1d).

This compound was prepared according to the general procedure described above. The following amounts of starting material and reagents were employed: 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 reaction mixture was stirred at 0° for 3 h and worked up as described above, including silica gel

column chromatography (2-propanol:NH_4OH:H_2O = 80:10:10→75:15:10) and RP-HPLC (30 mM aq TEAB:CH_3CN = 80:20→75:25, 16 ml/min), yielding title compound **1d** (98 mg, 47%) as a white solid. ^{1}H-NMR (500 MHz, D_2O): 6.45 (*dd*, *J* = 7.4, 4.0, 1H, H-1'); 4.55 (*m*, 1H, H-3'); 4.07 (*m*, 1H, H-4'); 4.0-3.92 (*m*, 1H, H-5'); 3.84-3.76 (*m*, 1H, H-5'); 2.70-2.50 (*m*, 3H, H-2' and α-CH_2); 2.34-2.26 (*m*, 1H, H-2'); 1.69-1.53 (*m*, 2H, β-CH_2); 1.35-1.14 (*m*, 10H, 5 x CH_2); 0.80 (*t*, *J* = 6.9, 3H, CH_3). ^{13}C-NMR (125 MHz, D_2O): 157.91, 149.82, 147.81, 85.31, 84.96 (*d*, J_{CP} = 7.5, 1C), 70.07, 65.09 (*d*, J_{CP} = 5.1, 1C), 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).

This compound was prepared according to the general procedure described above. The following amounts of starting material and reagents were employed: 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 reaction mixture was stirred at 0° for 3 h and worked up as described above, including silica gel column chromatography (2-propanol:NH_4OH:H_2O = 85:10:5→80:10:10→75:15:10) and RP-HPLC (30 mM aq TEAB:CH_3CN = 92:8→80:20, 16 ml/min), yielding title compound **2** (49 mg, 40%) as a white solid. ^{1}H-NMR (500 MHz, D_2O): 7.84 (*m*, 2H, ArH); 7.24 (*m*, 2H, ArH); 6.57 (*dd*, *J* = 7.2, 5.0, 1H, H-1'); 4.58 (*m*, 1H, H-3'); 4.09 (*m*, 1H, H-4'); 3.90-3.83 (*m*, 1H, H-5'); 3.82-3.75 (*m*, 1H, H-5'); 2.82-2.74 (*m*, 1H, H-2'); 2.35-2.27 (*m*, 1H, H-2'). ^{13}C-NMR (150 MHz, D_2O): 163.24 (*d*, J_{CF} = 247.0, 1C), 160.19, 156.59, 143.54, 130.71 (*d*, J_{CF} = 8.5, 2C), 129.08, 115.22 (*d*, J_{CF} = 21.9, 2C), 85.78, 85.17 (*d*, J_{CP} = 7.1, 1C), 71.52, 64.60 (*d*, J_{CP} = 3.8, 1C), 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-(5-(2'-deoxy-6-azauridine-5'-monophosphate))prop-2-ynyl) octanamide (3).**

This compound was prepared according to the general procedure described above. The following amounts of starting material and reagents were employed: 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 reaction mixture was stirred at 0° for 3 h and worked up as described above, including silica gel column chromatography (2-propanol:NH_4OH:H_2O = 85:10:5→80:10:10→75:15:10) and RP-HPLC (30 mM aq TEAB:CH_3CN = 85:15→80:20→65:35, 16 ml/min), yielding title compound **3** (16 mg, 17%) as a white solid. ^{1}H-NMR (500 MHz, D_2O): 6.51 (*t*, *J* = 6.0, 1H, H-1'); 4.59 (*m*, 1H, H-3'); 4.23 (*s*, 2H, α-CH_2); 4.10 (*m*, 1H, H-4'); 3.98-3.91 (*m*, 1H, H-5'); 3.88-3.80 (*m*, 1H, H-5'); 2.75-2.66 (*m*, 1H, H-2'); 2.35-2.25 (*m*, 1H, H-2'); 2.30 (*t*, *J* = 7.1, 2H, CH_2CO); 1.63 (*m*, 2H, C*H*$_2CH_2CO$); 1.30 (br. *s*, 4H, 2 x CH_2); 1.23 (br. *s*, 4H, 2 x CH_2); 0.81 (*t*, *J* = 6.8, 3H, CH_3). ^{13}C-NMR (150 MHz, D_2O): 177.40, 163.93, 154.79, 131.01, 91.79, 86.25, 85.28 (*d*, J_{CP} = 7.7, 1C), 74.86, 71.34, 65.04 (*d*, J_{CP} = 4.6, 1C), 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-(5-(2'-deoxy-6-azauridine-5'-monophosphate))3-oxopropyl)octanamide (4).**

This compound was prepared according to the general procedure described above. The following amounts of starting material and reagents were employed:

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 reaction mixture was stirred at 0° for 3 h and worked up as described above, including silica gel column chromatography (2-propanol:NH_4OH:H_2O = 85:10:5→80:10:10→75:15:10) and RP-HPLC (30 mM aq TEAB:CH_3CN = 85:15→80:20→75:25, 16 ml/min), yielding title compound **4** (24.6 mg, 22%) as a white solid. ^{1}H-NMR (600 MHz, D_2O): 6.49 (*dd*, *J* = 7.2, 4.7, 1H, H-1'); 4.54 (*m*, 1H, H-3'); 4.07 (*m*, 1H, H-4'); 3.94-3.97 (*m*, 1H, H-5'); 3.83-3.76 (*m*, 1H, H-5'); 2.70-2.64 (*m*, 1H, H-2'); 2.32-2.23 (*m*, 1H, H-2'); 2.28 (*t*, *J* = 7.4, 2H, NHCOC*H*$_2$); 1.57 (*m*, 2H, NHCOCH$_2$C*H*$_2$); 1.31-1.18 (br. *m*, 8H, 4 x CH_2); 0.81 (*t*, *J* = 6.9, 3H, CH_3). ^{13}C-NMR (150 MHz, D_2O): 204.97, 177.82, 158.31, 150.62, 141.96, 85.89, 85.32 (*d*, J_{CP} = 7.9, 1C), 71.26, 65.0 (*d*, J_{CP} = 4.2, 1C), 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 inhibition assays, Cloning, expression and purification of mycobacterial ThyX

This was done according to protocols described [22].

References

[1] S.E. Haydel, *Pharmaceuticals* **2010**, *3*, 2268.

[2] B. Petrini, S. Hoffner, *Int*. *J*. *Antimicrob*. *Agents* **1999**, *13*, 93.

[3] S.E. Dorman, *Nature Med*. **2007**, *13*, 295.

[4] J.M. FitzGerald, S. Houston, *Can*. *Med*. *Ass*. *J*. **1999**, *161*, 47.

[5] R. Rappuoli, A. Aderem, *Nature* **2011**, *473*, 463

[6] *WHO Progress Report 2011*. Geneva, World Health Organization, **2011**.

[7] A.A. Velayati, M.R. Masjedi, P. Farnia, P. Tabarsi, J. Ghanavi, A.H. ZiaZarifi, S.E. Hoffner, *CHEST* **2009**, *136*, 420.

[8] C. Saltini, *Resp*. *Med*. **2006**, *100*, 2085.

[9] E.K. Schroeder, O.N. de Souza, D.S. Santos, J.S. Blanchard, L.A. Basso, *Curr*. *Pharm*. *Biotechnol*. **2002**, 3, 197.

[10] L.P. Miller, J.T. Crawford, T.M. Shinnick, *Antimicrob*. *Agents Chemother*. **1994**, *38*, 805.

[11] Y. Zhang, M.M. Wade, A. Scorpio, H. Zhang, Z. Sun, *J. Antimicrob*. *Chemother*. **2003**, *52*, 790.

[12] K.N. Williams, C.K. Stover, T. Zhu, R. Tasneen, S. Tyagi, J.H. Grosset, E. Nuermberger, *Antimicrob*. *Agents Chemother*. **2009**, *53*, 1314.

[13] K. Andries, P. Verhasselt, J. Guillemont, H.W. Göhlmann, J.-M. Neefs, H. Winkler, J. Van Gestel, P. Timmerman, M. Zhu, E. Lee, P. Williams, D. de Chaffoy, E. Huitric, S. Hoffner, E. Cambau, C. Truffot-Pernot, N. Lounis, V. Jarlier, *Science* **2005**, *307*, 223.

[14] B. Zhou, Y. He, X. Zhang, J. Xu, Y. Luo, Y. Wang, S.G. Franzblau, Z. Yang, R.J. Chan, Y. Liu, J. Zheng, Z.-Y. Zhang, *Proc*. *Natl*. *Acad*. *Sci*. *USA* **2010**, *107*, 4573.

[15] D. Tondi, A. Venturelli, S. Ferrari, S. Ghelli, M.P. Costi, *J. Med. Chem*. **2005**, *48*, 913.

[16] K.M. Perry, E.B. Faumann, J.S. Finer-Moore, W.R. Montfort, G.F. Maley, F. Maley, R.M. Stroud, *Proteins* **1990**, 8, 315.

[17] H. Myllykallio, G. Lipowski, D. Leduc, J. Filee, P. Forterre, U. Liebl, *Science* **2002**, 297, 105.

[18] C.W. Carreras, D.V. Santi, *Annu. Rev. Biochem.* **1995**, *64*, 721.

[19] N. Agrawal, S.A. Lesley, P. Kuhn, A. Kohen, *Biochemistry* **2004**, *43*, 10295.

[20] E.M. Koehn, T. Fleischmann, J.A. Conrad, B.A. Palfey, S.A. Lesley, I.I. Mathews, A. Kohen, *Nature* **2009**, *458*, 919; E.M. Koehn, A. Kohen, *Arch. Biochem. Biophys.* **2010**, *493*, 96.

[21] J. Rengarajan, B.R. Bloom, E.J. Rubin, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 8327.

[22] M. Kögler, B. Vanderhoydonck, S. De Jonghe, J. Rozenski, K. Van Belle, J. Herman, T. Louat, A. Parchina, C. Sibley, E. Lescrinier, P. Herdewijn, *J. Med. Chem.* **2011**, *54*, 4847.

[23] U. Niedballa, H. Vorbrüggen, *Angew. Chem. Int. Ed.* **1970**, *9*, 461; H. Vorbrüggen, K. Krolilіewicz, U. Niedballa, *Liebigs Ann. Chem.* **1975**, 988; H. Vorbrüggen, C. Ruh-Pohlenz (Eds.) in: *Handbook of nucleoside synthesis*, Wiley-VCH Weinheim, **2001**.

[24] M. Hoffer, *Chem. Ber.* **1960**, *93*, 2777; V. Rolland, M. Kotera, J. Lhomme, *Synth. Comm.* **1997**, *27*, 3505.

[25] I.E. Dupont Passelaigue, J.F. Patoiseau, D. Junquero, Y. Rival, A. Delhon, *Fr. Demande* **2005**, 131 pp.

[26] P.K. Chang, *J. Org. Chem.* **1961**, *26*, 1118.

[27] J.N. Freskos, *Nucleosides Nucleotides* **1989**, *8*, 549.

[28] J. Asakura, M.J. Robins, *J. Org. Chem.* **1990**, *55*, 4928.

[29] M. Yoshikawa, T. Kato, T. Takenish, *Bull*. *Chem*. *Soc*. *Jpn*. **1969**, *42*, 3505.

[30] C. Savarin, L.S. Liebeskind, *Org*. *Lett*. **2001**, *3*, 2149.

[31] K. Sonogashira, *J*. *Organomet*. *Chem*. **2002**, *653*, 46.

[32] J.H. Hunter, R. Gujjar, C.K.T. Pang, P.K. Rathod, *PLoS One* **2008**, *3*, e2237; S.C. Kan, J.S. Liu, H.Y. Hu, C.M. Chang, W.D. Lin, W.C. Wang, W.H. Hsu, *Biochim*. *Biophys*. *Acta* **2010**, *1804*, 1751.

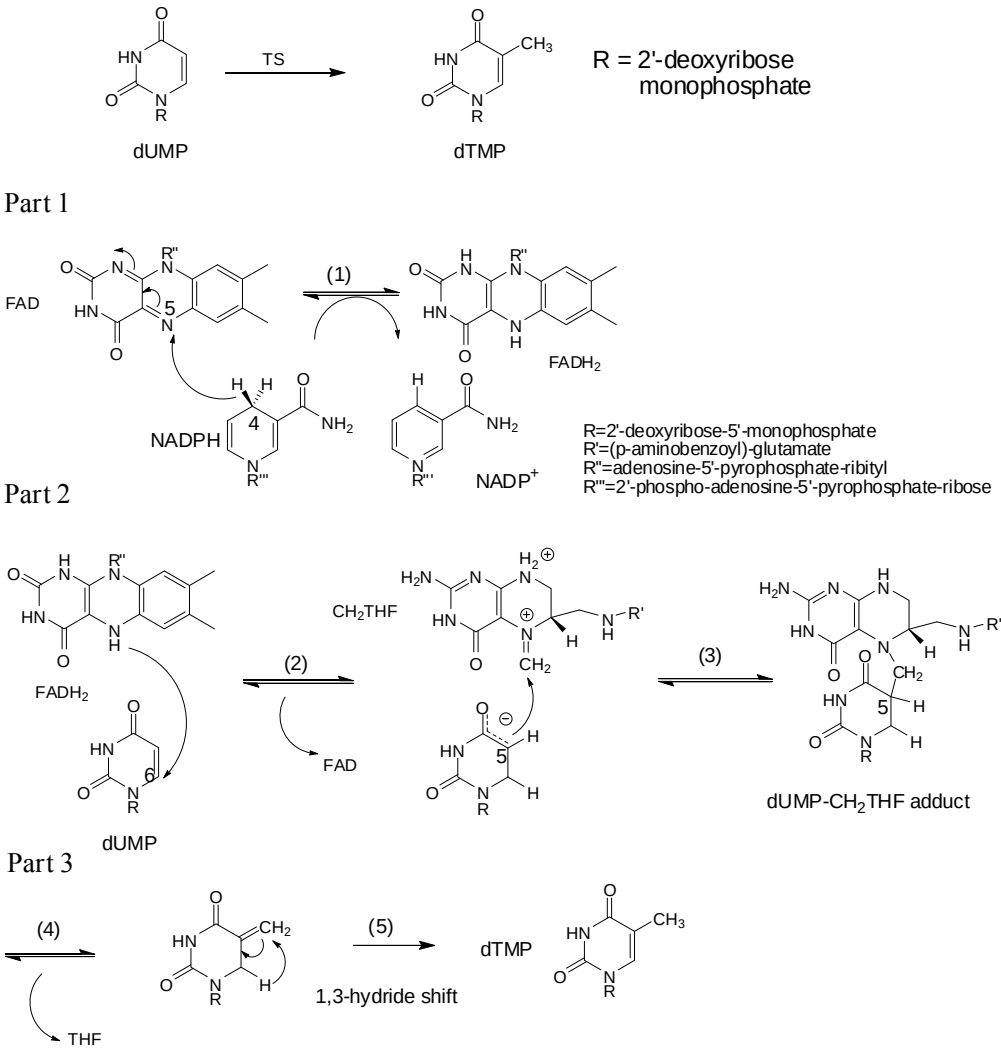
[33] P. Sampathkumar, S. Turley, J.E. Ulmer, H.G. Rhie, C.H. Sibley, W.G. Hol, J. Mol. Biol. **2005**, 352, 1091.

*Table 1: Inhibition of Mtb-ThyX and Mtb-ThyA by compounds **1a-d**, **2-4**.*

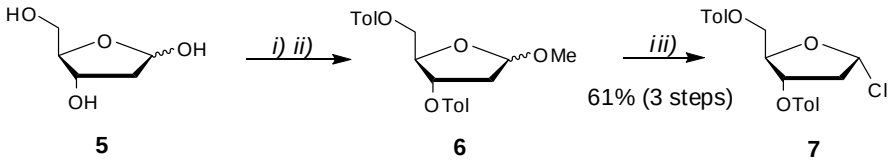
	ThyX-inhibition[a]		ThyA-inhibition[a]	
Compd	% inhibition (50 µM)	IC_{50} (µM)	% inhibition (50 µM)	IC_{50} (µM)
5-FdUMP[b]	95.1	0.29	100	0.57
1a	33.5	> 50	0.99	> 50
1b	-9.3	> 50	8.78	> 50
1c	-42.8	> 50	20.04	> 50
1d	5.7	> 50	3.67	> 50
2	-14.1	> 50	8.65	> 50
3	40.9	> 50	13.18	> 50
4	-12.6	> 50	14.71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

[a]values are means of three independent experiments, [b]5-FdUMP used as positive control

Scheme 1 *Biochemical reaction mechanism investigated by Koehn et al. (see reference 20).*

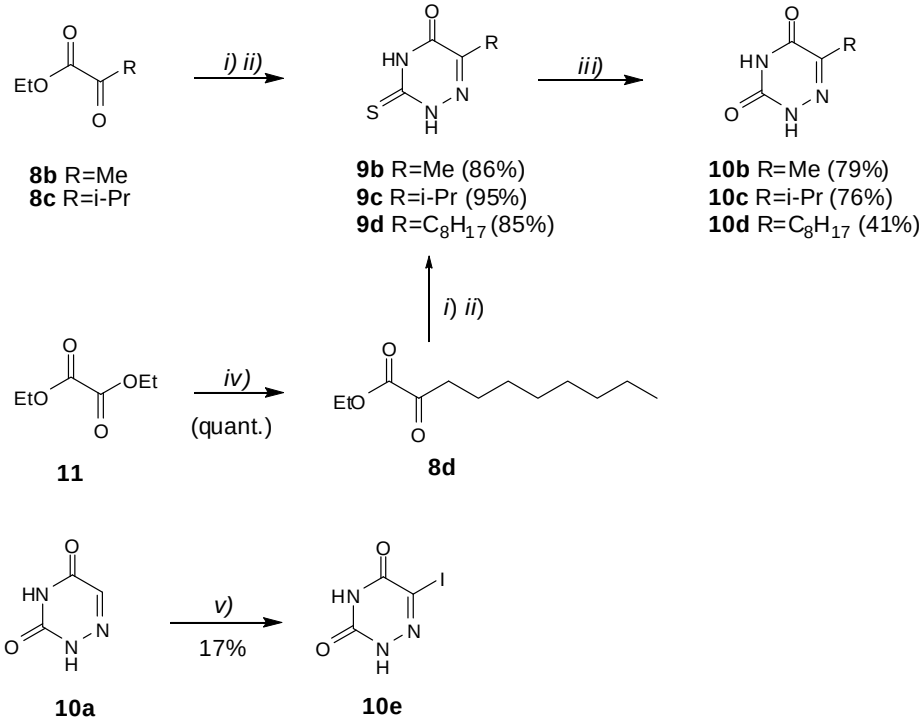


Scheme 2



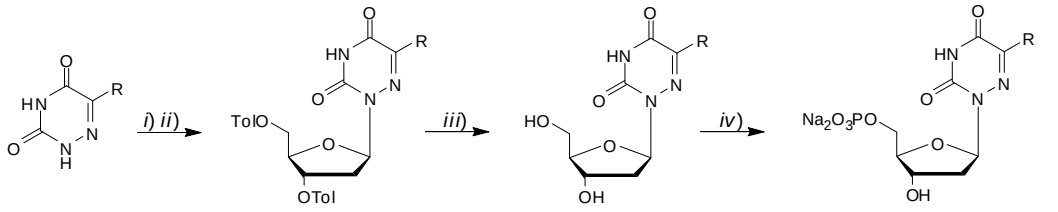
i) HCl/MeOH, r.t. *ii*) *p*-TolCl, pyr, r.t. *iii*) HCl/AcOH, r.t.

Scheme 3



i) thiosemicarbazide, EtOH, 90°. *ii*) NaOH, H_2O, reflux. *iii*) 35% aq H_2O_2, 1 M NaOH, rt. *iv*) $C_8H_{17}MgBr$, THF/Et_2O, -60°. *v*) I_2, KI, NaOH, H_2O, reflux.

Scheme 4



10a R=H
10b R=Me
10c R=i-Pr
10d R=C_8H_{17}

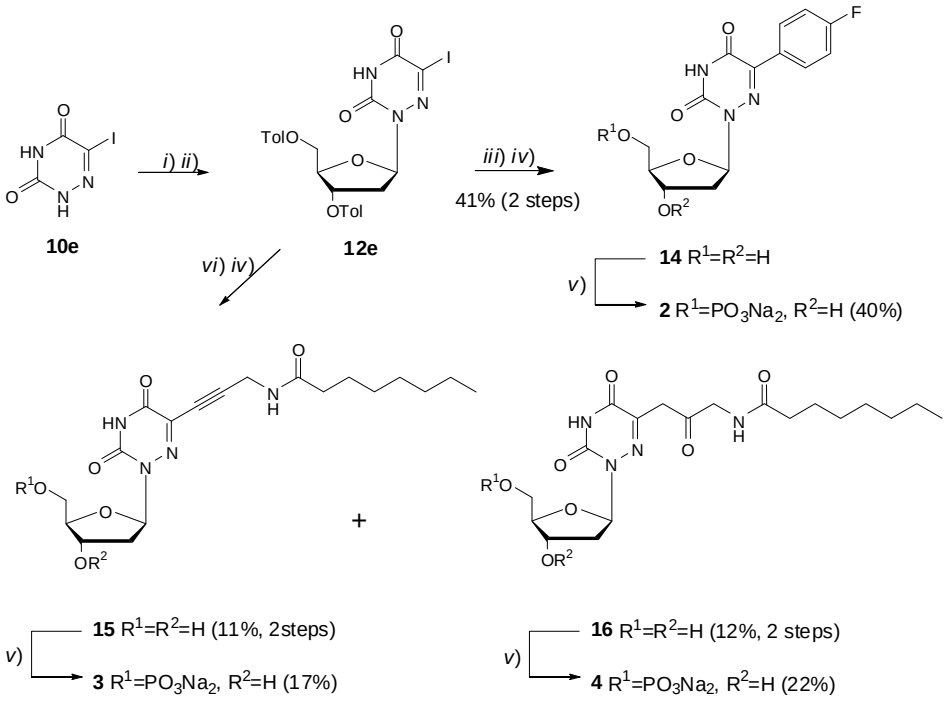
12a R=H (58%)
12b R=Me (73%)
12c R=i-Pr (43%)
12d R=C_8H_{17} (66%)

13a R=H
13b R=Me
13c R=i-Pr
13d R=C_8H_{17}

1a R=H (40%)
1b R=Me (53%)
1c R=i-Pr (17%)
1d R=C_8H_{17} (47%)

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

Scheme 5



10e

12e

41% (2 steps)

14 $R^1=R^2=H$

2 $R^1=PO_3Na_2$, $R^2=H$ (40%)

15 $R^1=R^2=H$ (11%, 2steps)

3 $R^1=PO_3Na_2$, $R^2=H$ (17%)

16 $R^1=R^2=H$ (12%, 2 steps)

4 $R^1=PO_3Na_2$, $R^2=H$ (22%)

i) HMDS, $(NH_4)_2SO_4$, reflux. *ii*) chlorosugar **7**, CuI, TMSCl, $CHCl_3$, r.t. *iii*) 4-fluorophenylboronic acid, $Pd(PPh_3)_4$, CuTC, THF, 50°. *iv*) 0.1 M NaOMe, 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, NEt_3, DMF, r.t.

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

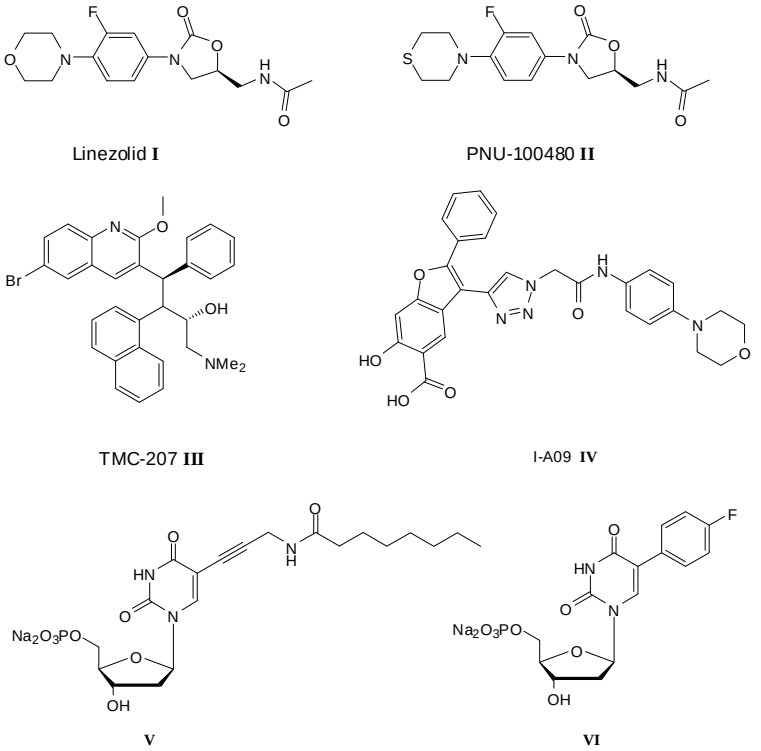


Figure 2 *Structures of synthesized 6-aza dUMP analogues*.

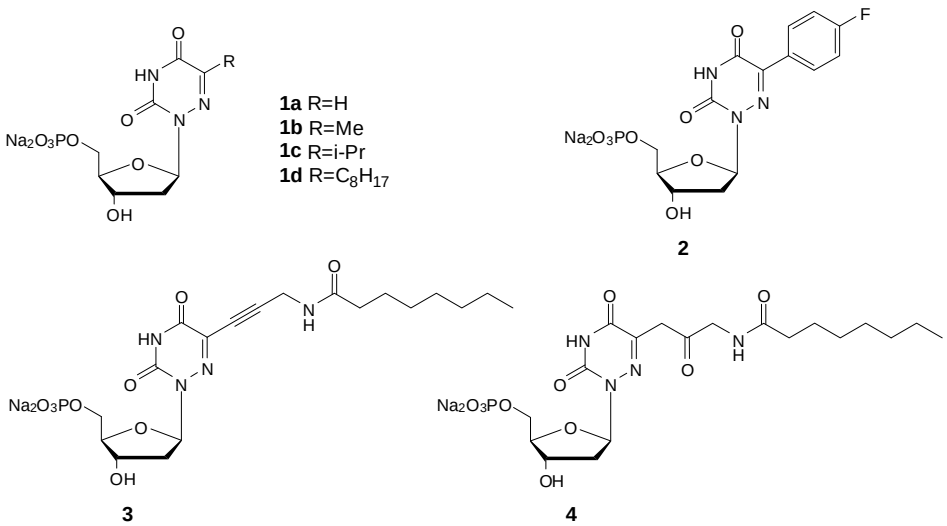


Figure 3 *^{13}C- spectrum of compound **4** in D_2O (left) and (D_6)-DMSO (right).*

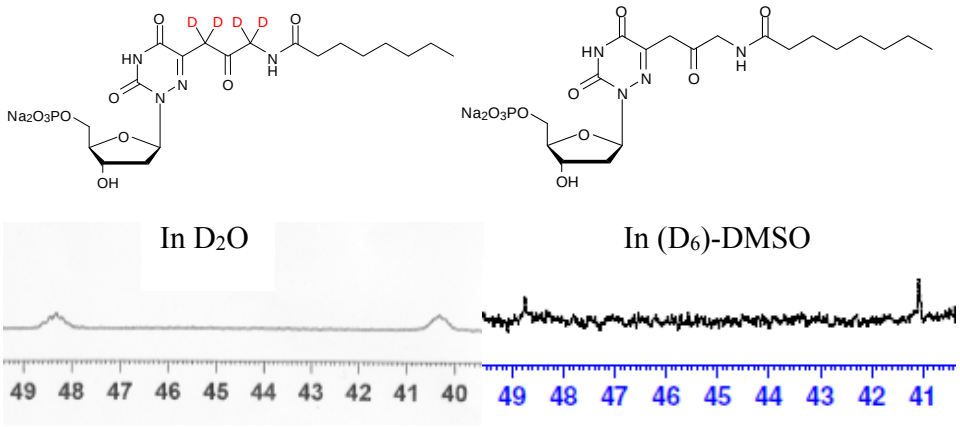
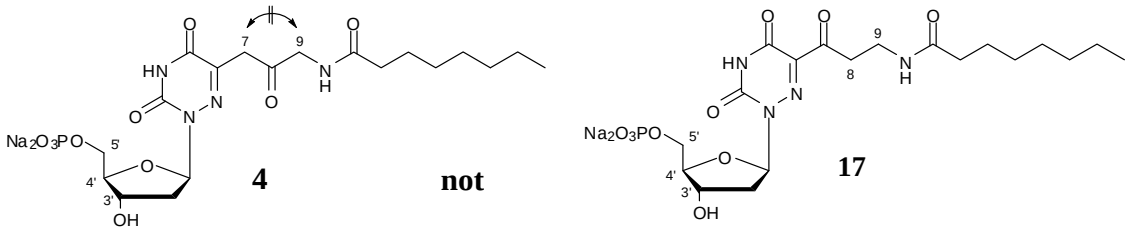


*Figure 4. COSY spectrum of compound **4** in (D_6)-DMSO.*



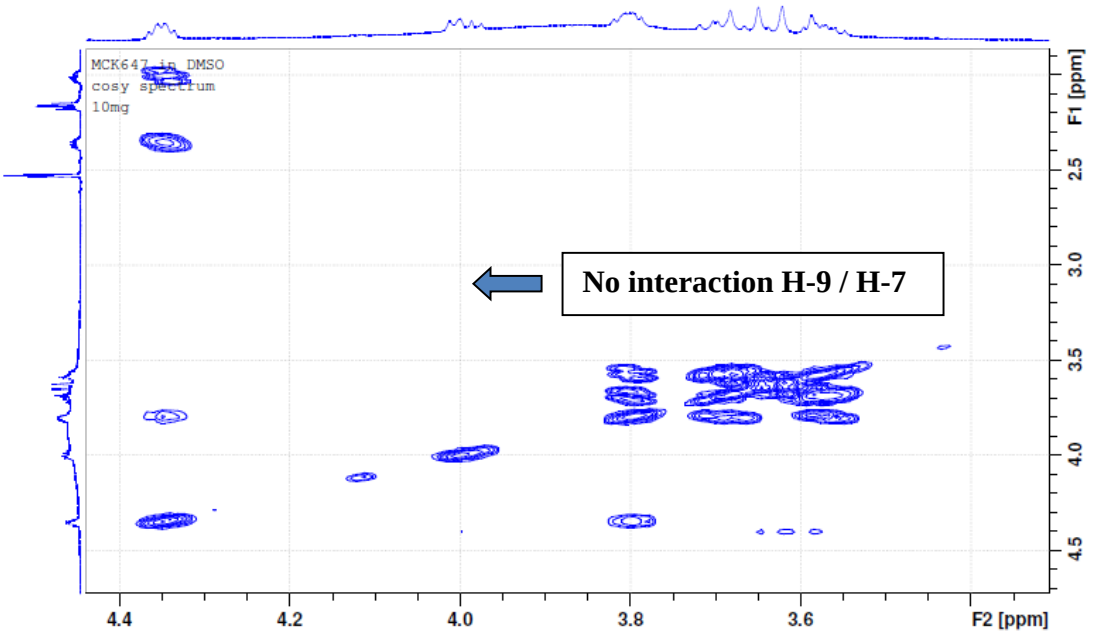
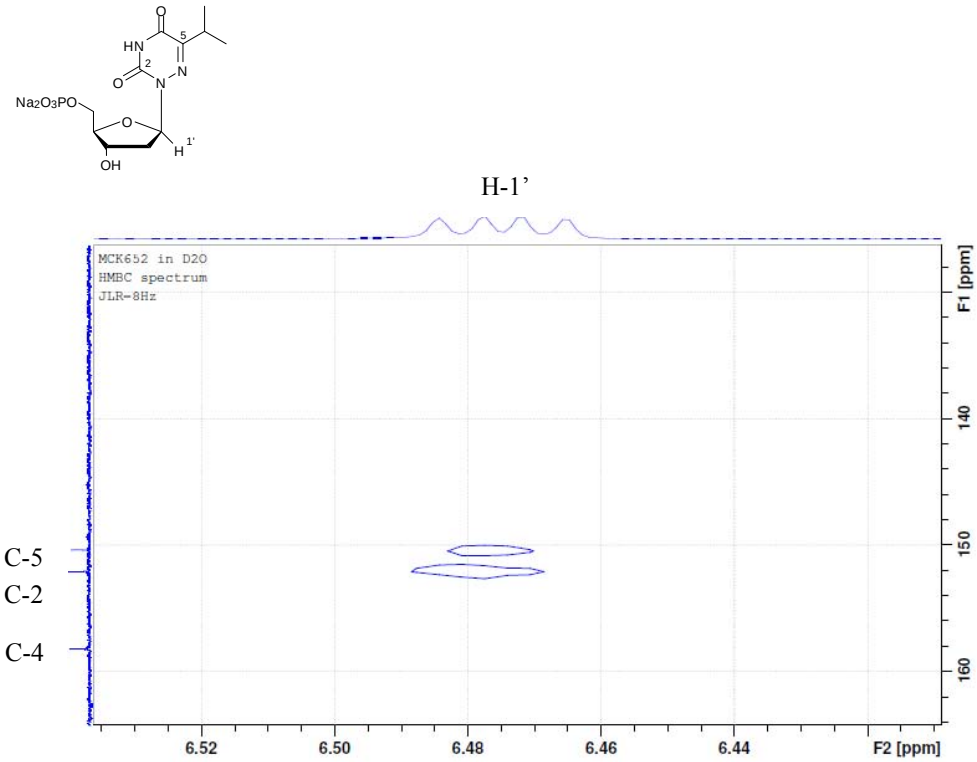


Figure 5 *HMBC spectrum of compound* ***1c***.

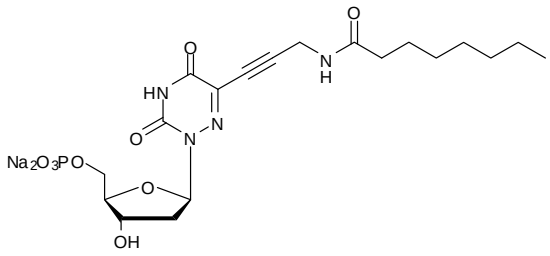


Abbreviations:

AcOH, Acetic acid; CH_2THF, Methylenetetrahydrofolic acid; CuTC, Copper(I)-thiophene carboxylate; dba, dibenzylideneacetone; DMF, Dimethylformamide;

DMSO, Dimethylsulfoxide; DOTS, Directly observed treatment short; dTMP, 2'-Deoxythymidine-5'-monophosphate; dUMP, 2'-Deoxyuridine-5'-monophosphate; AcOEt, Ethyl acetate; EtOH, Ethanol; FAD, Flavin adenine dinucleotide; HIV, Human Immunodeficiency Virus; HMDS, 1,1,1,3,3,3-Hexamethyldisilazane; HRMS, High-resoln. mass spectrometry, IC_{50}, Half maximal (50%) inhibitory concentration; MDR-TB, Multi drug-resistant TB; MeOH, Methanol; mPTPB, mycobacterial protein tyrosine phosphatase B; NADPH, Nicotinamide adenine nucleotide phosphate hydride; NaOMe, Sodium methoxide; NEt_3, Triethylamine; RP-HPLC, Reversed-phase High performance liquid chromatography; r.t., room temperature; SAR, Structure-activity relationship; TB, Tuberculosis; TEAB, Triethylammonium bicarbonate; THF, Tetrahydrofuran; ThyA, Thymidylate synthase; ThyX, Flavin-dependent thymidylate synthase; TMSCl, Trimethylsilyl chloride; TS, Thymidylate Synthase; WHO, World Health Organization; XDR-TB, Extensively drug-resistant TB.

Graphical Abstract



40% inhibition against mycobacterial ThyX at 50μM