Bioorganic & Medicinal Chemistry 21 (2013) 257–268

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and evaluation of 5′-modified thymidines and 5-hydroxymethyl-2′-deoxyuridines as *Mycobacterium tuberculosis* thymidylate kinase inhibitors

Kiran S. Toti [a], Frederick Verbeke [a], Martijn D.P. Risseeuw [a], Vladimir Frecer [b], Hélène Munier-Lehmann [c,d], Serge Van Calenbergh [a,*]

[a] Laboratory for Medicinal Chemistry, Faculty of Pharmaceutical Sciences (FFW), Ghent University, Harelbekestraat 72, 9000 Ghent, Belgium
[b] Department of Physical Chemistry of Drugs, Faculty of Pharmacy, Comenius University, 83232 Bratislava, Slovakia
[c] Institut Pasteur, Unité de Chimie et Biocatalyse, 28 rue du Dr Roux, 75724 Paris Cedex 15, France
[d] CNRS UMR3523, 28 rue du Dr Roux, France

ARTICLE INFO

Article history:
Received 23 August 2012
Revised 17 October 2012
Accepted 18 October 2012
Available online 5 November 2012

Keywords:
Mycobacterium tuberculosis
Thymidine monophosphate kinase
Inhibitor
Nucleoside analogues
5-Hydroxymethyl pyrimidines
Phosphate bioisosteres

ABSTRACT

We report the synthesis of 5′-modified thymidines (**16**, **18**, **21**, **23**) and 5,5′-bis-substituted 2′-deoxyuridine analogues (**30**, **47**) as inhibitors of thymidine monophosphate kinase of *Mycobacterium tuberculosis* (TMPK*mt*). These analogues were evaluated for their capacity to inhibit TMPK*mt* and solely two 5′-modified thymidines were found to possess moderate inhibitory activity. In addition, a feasibility study of protecting groups for the 5-CH_2OH moiety of 2′-deoxyuridines is described that enables to introduce the desired 5′-modification.

1. Introduction

Tuberculosis (TB) is responsible for more than 2 million human deaths annually. The emergence of multi-drug and extremely drug resistant TB strains asks for new drugs preferably acting on yet unaddressed targets.[1] The knowledge of the TB genome should enable to reveal new metabolic pathways and targets, which may fuel the discovery process of antituberculosis drugs.[2] Phosphorylation of nucleosides is a prerequisite for DNA synthesis and the enzymes in charge are crucial for cell growth. Thymidine monophosphate kinase is situated at the junction of the de novo and salvage pathways for thymidine triphosphate (dTTP) synthesis. TMPK is the last specific enzyme in these pathways and has been recently validated in vivo as an antibacterial target.[3] We have focused our effort on the TMPK of *Mycobacterium tuberculosis* (TMPKmt) for generating anti-TB drugs. Previously, we designed a number of promising TMPK*mt* inhibitors such as the bicyclic inhibitor **1** and the 5′-thiourea-substituted α-thymidine analogue **2** (Fig. 1).[4] Furthermore, we showed that hydroxylation of the 5-methyl group of the TMP substrate (**3**) resulted in a moderately potent TMPK*mt* inhibitor instead of substrate.[5] Mainly based on inhibitory data reported by our group, Frecer et al. recently reported on the combinatorial design and structure-based in silico screening of a virtual focused library of bicyclic thymidine analogs in an effort to identify more potent TMPK*mt* inhibitors.[6] In their study they used the three dimensional structure of TMPK*mt* complexed with 5-hydroxymethyl-dUMP (**3**) to develop a QSAR model, to parameterize a target-specific scoring function for TMPK*mt* and to select virtual hits which display the highest predicted binding to the target. Compound **4** emerged as one of the best analogues. The binding of **4** to TMPK*mt* is predicted to be similar to that of **1**. Beside the fact that it is a carba-nucleoside contains a sulfondiamide group replacing the thiourethane moiety of the condensed ring of **1**, **4** differs from **1** by the presence of a 5-CH_2OH group, known to possibly make an extra hydrogen bond with a water molecule (W12) or with the side chain of Ser99, and a 5′-(*N*-methylsulfamoyl)methyl moiety. The latter fragment, which replaces the phosphate moiety present in TMP, was anticipated to favorably interact Arg95, Arg153 and especially with the magnesium ion. Alternative phosphate isosters suggested in the Frecer publication are a 5-tetrazolylmethyl and methylsulfonylmethyl group. In this pilot study we decided to explore the importance of the proposed 5-CH_2OH and 5′-modifications for TMPK*mt* inhibition.

* Corresponding author. Tel.: +32 9 264 81 24; fax: +32 9 264 81 46.
E-mail address: serge.vancalenbergh@ugent.be (S. Van Calenbergh).

0968-0896/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2012.10.018

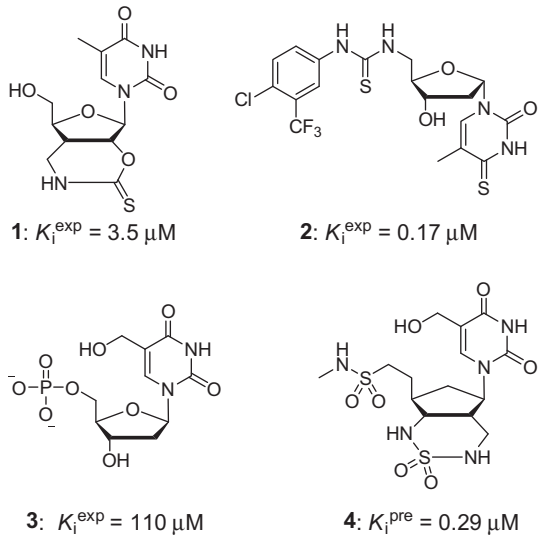


Figure 1. Selected promising TMPK*mt* inhibitors designed previously in our laboratory (**1**, **2**, and **3**)[4,5] or predicted by a QSAR model (**4**).[6] K_i^{exp} and K_i^{pre} indicate experimental and predicted K_i values, respectively. The K_m value for dTMP is 4.5 μM.

2. Results and discussion

2.1. Chemistry

We envisioned synthesizing the target molecules using a Wittig–Horner reaction to introduce the desired 5′-modifications as a key step. For this purpose two non-commercial Horner reagents were self-made (Scheme 1). Diethyl((methylsulfonyl) methyl)phosphonate **6** was prepared according to known procedures,[7] while Horner reagent **9** was obtained by reacting half an equivalent of chlorodiethylphosphate with bis-lithiated **8.**[8,9] 3′-Silylated thymidine **12** was produced by a selective cleavage of the primary TBDMS group on **11**.[10,11]

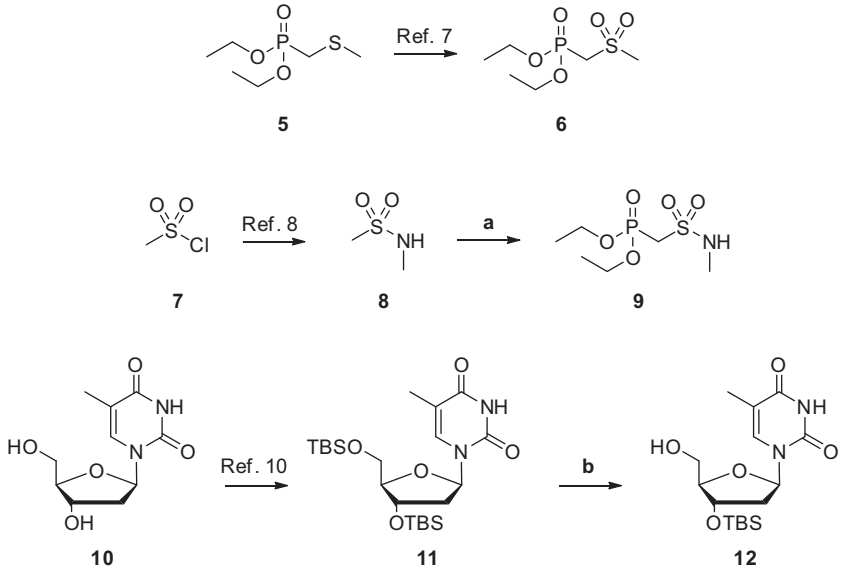
To synthesize the 5′-modified thymidines **16**, **18**, **21** and **23** (Scheme 2), the 5′-OH group of **12** was oxidized using Dess–Martin periodinane and the resulting aldehyde **13** was reacted with the proper Wittig–Horner reagents to give **14**, **17** and **19** in moderate yields. Methylsulfone **16** was obtained by Pd catalyzed hydrogenation of **14**, followed by the removal of TBDMS group. The conjugated double bond in **17** was reduced using $NaBH_4/NiCl_2$[12] and the product was desilylated using TBAF affording **18**. Conjugate reduction of nitrile **19** was achieved with $NaBH_4$ in a pyridine–methanol mixture at elevated temperature.[13] Deprotection of **20** afforded the 6′-nitrile analogue **21** in 76% yield. A cycloaddition between nitrile **20** and $TMSN_3$ in the presence of dibutyltinoxide gave **22** in moderate yield.[14] Even though the conversion of **22** to **23** was complete (by LCMS), the isolated yield is lowered by the elaborate purification necessary to isolate the product.[15]

Hydroxymethylation at position 5 of dU (**24**) of pyrimidine is reported. However, selective protection of this hydroxyl group appears difficult.[16] An attempt to hydroxymethylate the 5-position of **25** failed, possibly due to the low solubility of the substrate in formalin-triethylamine system and the lability of the TBDMS groups under the harsh reaction conditions. Hence, we decided to introduce the 5-CH_2OH group after modifying the 5′-position in 2′-deoxyuridine (Scheme 3). As a model substrate for this strategy, compound **29** was synthesized smoothly using similar transformations as before. Unfortunately, reaction of **29** with formaldehyde at elevated temperature was very slow with only traces of the desired product formed after several days, while under microwave conditions, the starting material degraded.

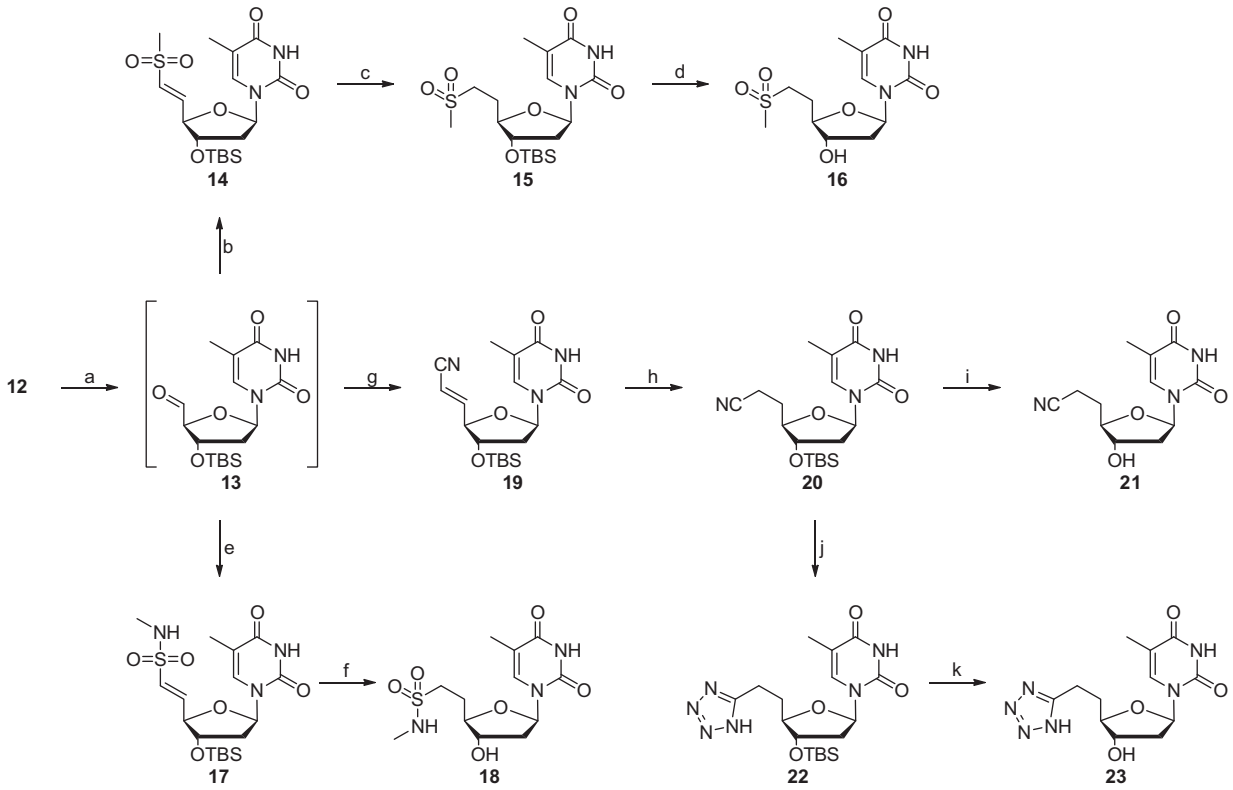
Because of this setback, we decided to start from compound **31**, obtained via the benzylic bromide oxidation as reported by Grover et al.[10] (Scheme 4). Selective primary desilylation of **31** gave **32**. Attempted successive Dess–Martin and Wittig–Horner reactions involving two different Horner reagents failed to give the desired products.

At this point we chose to look for a suitable protecting group for the 5-hydroxymethyl moiety that is compatible with the reaction conditions used to modify the 5′-position and can also be removed under mild conditions. Towards this end 5-hydroxymethyl derivative **33** was synthesized according to a literature procedure.[18]

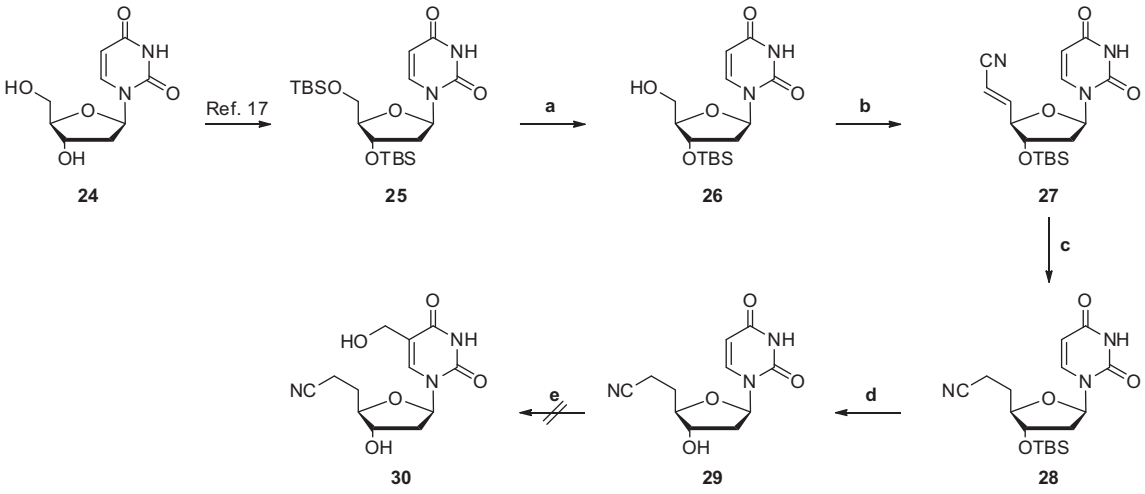
BOM derivatization of **33** to **34** proceeded in 37% yield (Scheme 5). Since the desired product also features a benzylic hydroxyl group, we ran a model hydrogenation reaction on **34** with palladium as catalyst. Unsurprisingly, the reaction mainly afforded thymidine **11**, besides minor amounts (24%) of **12**. Next, the use of a 2-(trimethylsilyl)ethoxymethyl (SEM) protecting group, known



Scheme 1. Synthesis of Horner reagents **6** and **9**, and intermediate **14**. Reagents and conditions: (a) THF, BuLi, −78 °C, 1 h, diethylchlorophosphate (0.5 equiv), −78 °C → 0 °C, 1 h, 65%; (b) THF, pyridine, HF-pyridine, 0 °C, 1 h, 0 °C → rt, 1 h, 36%.



Scheme 2. Synthesis of the 5′-modified thymidines. Reagents and conditions: (a) CH_2Cl_2, Dess–Martin periodinane, rt, 4 h; (b) THF, **6**, BuLi, −78 °C, 15 min, −78 °C, 1 h, rt, 18 h, 20% from **12**; (c) H_2, Pd-C, MeOH, 4 h, rt; (d) THF, TBAF, 40 °C, 15 h, 20% over two steps; (e) THF, **9**, BuLi, −78 °C, 1 h, −78 °C, 1 h, rt, 18 h, 48% from **12**; (f) (i) MeOH-THF (5:1), $NiCl_2$, $NaBH_4$, 0 °C, 1 h; (ii) THF, TBAF, 40 °C, 1 h, 12% over two steps; (g) THF, cyanomethyltriphenylphosphonium chloride, BuLi, 0 °C, 30 min, 0 °C → rt, 18 h, 57% from **12**; (h) pyridine-MeOH (3:1), $NaBH_4$, 120 °C, 4 h, 53%; (i) THF, TBAF, rt, 4 h, 76%; (j) toluene, $TMSN_3$, Bu_2SnO, 110 °C, 4 h, 32%; (k) THF, TBAF, rt, 4 h, 34%.

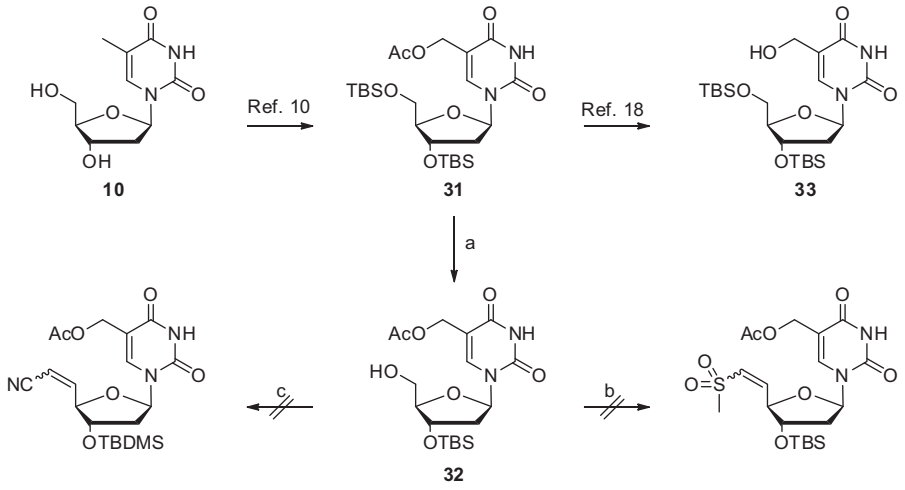


Scheme 3. Attempted hydroxymethylation route to **30**. Reagents and conditions: (a) THF, pyridine, HF-pyridine, 0 °C, 1 h, 0 °C → rt, 1 h, 53%; (b) (i) CH_2Cl_2, Dess–Martin periodinane, rt, 4 h; (ii) THF, cyanomethyltriphenylphosphonium chloride, BuLi, 0 °C, 30 min, 0 °C → rt, 18 h, 51% over two steps; (c) H_2, Pd-C, MeOH, 4 h, rt; (d) THF, TBAF, rt, 4 h, 73%; (e) paraformaldehyde, H_2O, TEA, 100 °C, 3 days or paraformaldehyde, H_2O, TEA, continuous irradiation of microwave from rt → 150 °C in 3 min, 10 min at 150 °C. (See above mentioned reference for further information).

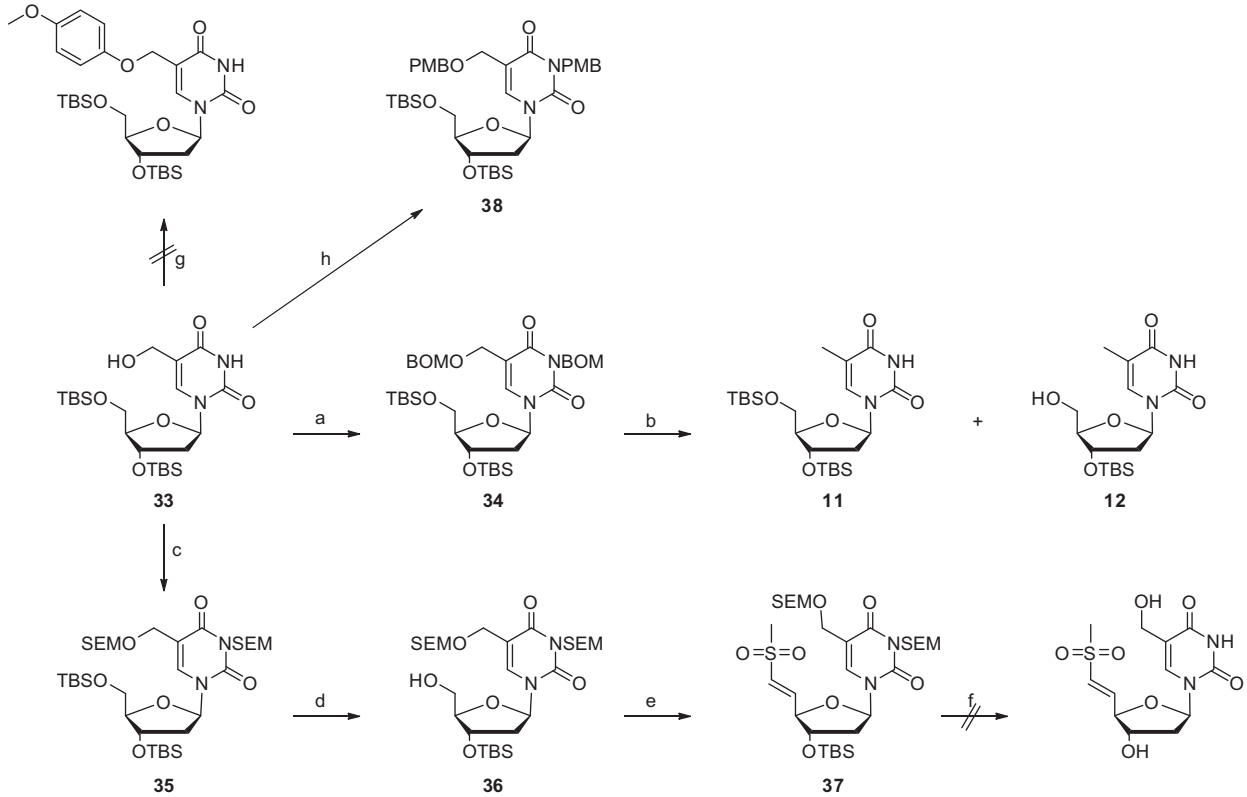
to be cleavable under mild conditions, was investigated. Compound **33** was converted to **35** in moderate yield. Selective removal of the primary TBS gave **36**, which on DMP oxidation followed by Horner reaction gave **37**. Unfortunately, attempts to remove both SEM groups with TBAF in anhydrous THF at elevated temperature failed.

Attempts to protect **33** as 4-methoxyphenol (PMP) ether under the Mitsunobu conditions led to degradation of the starting material. Conditions used to protect **33** with two 4-methoxybenzyl (PMB) groups afforded **38** only in low yields (13%), mainly because of simultaneous formation of compounds with three PMB & one TBS group and four PMB groups, leaving these protection methods unattractive.

These problems finally led us to protect the 5-CH_2OH group of **33** as pivaloate ester in good yield (Scheme 6). A series of routine transformations was used to convert **39** into the acrylonitrile **41** in acceptable yields. Several conditions were evaluated to reduce the double bond in **41**, including H_2–Pd–C, $NaBH_4$ in pyridine



Scheme 4. Attempted 5′-derivatization of acetyl protected 2′-deoxy-5-hydroxymethyluridine. Reagents and conditions: (a) anhyd THF, pyridine, HF-pyridine, 0 °C, 1 h, 0 °C → rt, 1 h, 33%; (b) (i) anhyd CH_2Cl_2, Dess–Martin periodinane, rt, 4 h; (ii) anhyd THF, **6**, BuLi, −78 °C, 15 min, −78 °C, 1 h, rt; (c) (i) anhyd CH_2Cl_2, Dess–Martin periodinane, rt, 4 h; (ii) anhyd THF, cyanomethyltriphenylphosphonium chloride, BuLi, 0 °C, 30 min, 0 °C → rt.

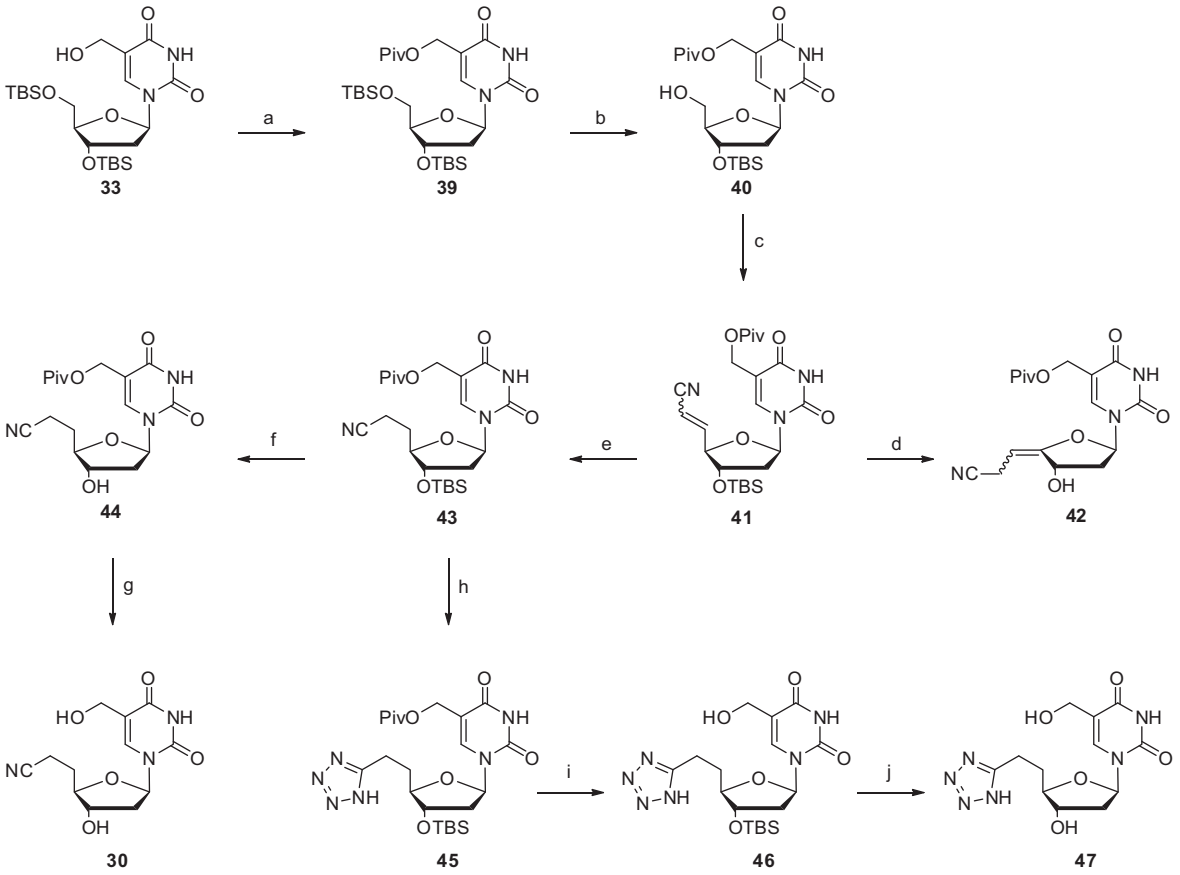


Scheme 5. Screening viable protecting group for 5-hydroxymethyl-2′-deoxyuridine. Reagents and conditions: (a) DMF, DIPEA, BOM-Cl, 0 °C → rt, 16 h, 37%; (b) MeOH, H_2, Pd-C; (c) CH_2Cl_2, DIPEA, SEM-Cl, 40 °C, 6 h, 50%; (d) THF, pyridine, HF-pyridine, 0 °C, 1 h, 0 °C → rt, 1 h, 38%; (e) (i) CH_2Cl_2, Dess–Martin periodinane, rt, 4 h; (ii) THF, **6**, BuLi, −78 °C, 15 min, −78 °C, 1 h, rt, 16 h, 29% over two steps; (f) THF, TBAF, Δ; (g) THF, DEAD, triphenylphosphine, PMP, 0 °C → rt; (h) DMF, PMB-Cl, NaH, 0 °C → rt, 4 h, 13%.

and $NaBH_4$–$NiCl_2$ in MeOH–THF. Unfortunately, all these methods led to deoxygenation of the 5-CH_2OH group and formation of a thymine base as a predominant side product. Attempts to remove the pivaloyl prior from **41** with tetrabutylammmonium hydroxide gave undesired product **42**. Hydrogenation using platinum on carbon afforded **43** in 70% yield. Subsequent removal of the TBDMS group with TBAF and the pivaloyl group with sodium methoxide gave **30** in excellent yield. Azidotrimethylsilane mediated cycloaddition of nitrile **43** gave **45** in 74% yield. Removal of the remaining protecting groups with NaOMe in methanol and NH_4F in methanol allowed obtaining **47** in excellent yield. For the desilylation NH_4F proved to be superior to TBAF with regard to purification of the final product.

2.2. Biological results

The capacity of compounds **16**, **18**, **21**, **23**, **30** and **47** to inhibit TMPK*mt* was assessed via a spectrophotometric binding assay (K_i) as previously described.[4] Compound **21** (K_i = 48 μM) and **23** (K_i = 70 μM) showed the highest activity, while compounds **16**



Scheme 6. Synthesis of the 5′-modified 2′-deoxy-5-hydroxymethyluridine analogs **30** and **47**. Reagents and conditions: (a) pyridine, DMAP, Piv-Cl, rt, 18 h,74%; (b) THF, pyridine, HF-pyridine, 0 °C, 1 h, 0 °C → rt, 1 h, 59%; (c) (i) CH_2Cl_2, Dess–Martin periodinane, rt, 4 h; (ii) anhyd THF, cyanomethyltriphenylphosphonium chloride, −78 °C, BuLi, 30 min, −78 °C → rt, 18 h, 82% over two steps; (d) THF, Bu_4NOH, rt, 5 h, 40%; (e) MeOH, H_2, Pt-C, rt, 4 h, 70%; (f) THF, TBAF, rt, 4 h, 90%; (g) 0.5 M NaOMe in MeOH, rt, 3 h, 95%; (h) toluene, $TMSN_3$, Bu_2SnO, 110 °C, 4 h, 74%; (i) 0.5 M NaOMe in MeOH, rt, 3 h, 92%; (j) NH_4F, MeOH, 50 °C, 2 days, 87%.

(340 μM) and **18** (240 μM) were comparatively less active. Surprisingly, compounds **30** and **47**, in which the favorable 5′-modifications were combined with a 5-CH_2OH modification of the nucleobase, failed to inhibit the enzyme at the highest concentration tested (2.6 and 2.4 mM, respectively). Clearly, introduction of the 5-hydroxymethyl group jeopardized the binding of 5′-modified analogues to TMPK*mt*.

2.3. Discussion and conclusions

In short, a small series of 5′-modified thymidine analogues was synthesized and evaluated as TMPK*mt* inhibitors. The analogues in which the 5′-hydroxyl group was replaced by an acetonitrile or a 5′-tetrazolylmethyl moiety proved capable of inhibiting the target enzyme with substantial affinity.

Hence we developed a synthetic route that allowed combining these 5′-modifications with a hydroxymethyl moiety at position 5 of the pyrimidine base. Given the recent interest in the phenomenon of hydroxymethylation of pyrimidine bases in mammalian genomes, the successful synthetic strategy, featuring pivaloyl protection of the 5-CH_2OH group prior to 5′-modification, may find future application in the synthesis of other 5-hydroxymethyl-2′-deoxyuridine tool compounds.

It is rather unfortunate that enzyme assays indicated that combination of both modifications did not reinforce the TMPK*mt* binding affinity and led to very weak inhibitors. A possible explanation of the observed drop in the inhibitory potencies of the 5-CH_2OH substituted compounds may be related to an elevated steric strain in the bisubstituted analogs. A superposition of the models of inhibitors **23** and **47** at the active site of TMPK*mt* (Fig. 2) shows that the 5′-tetrazolylmethyl moiety is firmly anchored in a polar cavity formed by the charged residues Asp9, Arg160, Asp163, and Glu166 and coordinated by the Mg^{2+} ion and a structural water molecule. However, the thymidine ring shows a higher degree of deviation between the two inhibitors, while the 5-CH_2OH group of **47** does not seem to reach up to an ideal stabilizing position. As shown on Figure 2, the oxygen of the 5-CH_2OH group is oriented mainly by the interactions with the Arg74 and Phe70 residues into a position where it directs its H atom towards the hydrogen of C_β of Phe36 with a relatively short distance of only 1.85 Å (red line in Fig. 2). This leads to an electrostatic repulsion, which may be responsible for the observed weak inhibitory potency of **47**. In the crystal structure of TMPK*mt* co-crystallyzed with 5-CH_2OH dUMP[5] the 5-hydroxymethyl group of dUMP is stabilized by hydrogen bonding interactions with the guanidine group of Arg74 and nitrogen atom of Pro37. Still, this does not exclude that a 2′,3′-α-fused cyclic sulfuryldiamide substituent may show synergistic inhibitory effects with a 5-CH_2OH group, since conformational restriction by a fused ring may very well change the topology of the molecular interactions with the enzyme.

3. Experimental

All reagents were from standard commercial sources and of analytical grade. Dry solvents were obtained directly from commercial sources and stored on molecular sieves. All reactions were carried out under argon atmosphere unless specified otherwise. Precoated Merck silica-gel F254 plates were used for TLC; spots

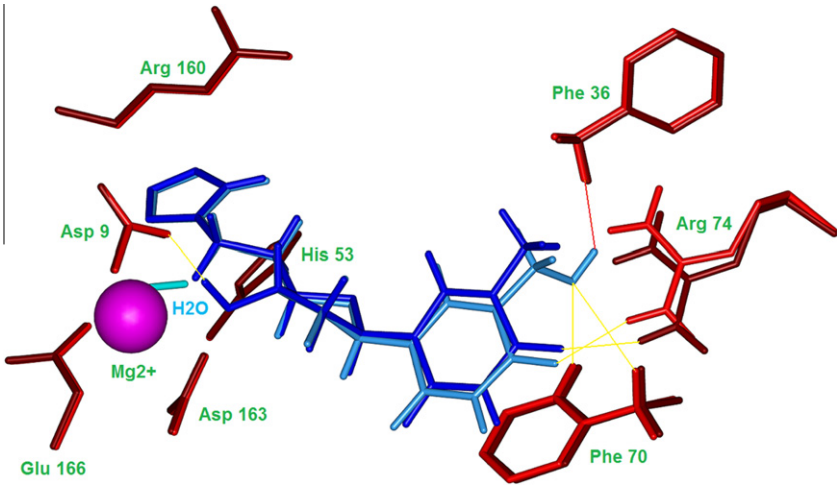


Figure 2. Comparison of structure and interactions of models of **23** (dark blue) and **47** (light blue) superimposed at the active site of TMPK*mt*. Side chains of selected active site residues are shown in stick representations. Majority of hydrogen atoms were omitted for better clarity. Structures of the enzyme-inhibitor complexes were obtained by flexible docking and refinement of the model ligands into the crystal structure of TMPK*mt* co-crystallyzed with 5-CH_2OH dUMP (PDB entry code 1MRS[5]). For method details see Ref. 6

were examined under ultraviolet light at 254 nm and further visualized by sulphuric acid–anisaldehyde spray. Column chromatography was performed on silica gel (200–400 mm, 60 Å). NMR spectra were recorded on a Varian Mercury 300 MHz spectrometer. Chemical shifts are given in ppm (δ), calibrated to the residual solvent signals or TMS. Exact mass measurements were performed on a Waters LCT PremierXETM time of flight (TOF) mass spectrometer equipped with a standard electrospray ionization (ESI) and modular LockSpray TM interface. Samples were infused in a CH_3CN/water (1:1 v/v) mixture at 10 mL/min. The microwave reactions were carried out in Milestone MicroSYNTH Advanced Microwave Synthesis Lab station, equipped with 2×800 W magnetrons (effective maximum output 1500 W pulsed/continuous), an optical fiber temperature sensor, a pressure sensor, under continues power mode in a closed PTFE vessel. A temperature of 25 ± 3 °C is referred to as 'room temperature, rt' throughout the manuscript. NMR signals of sugar protons and carbons are indicated with a prime, and signals of base protons and carbons are given without a prime.

3.0.1. Diethyl ((*N*-methylsulfamoyl)methyl)phosphonate (9)

To a solution of **8** (1.0 g, 9.1 mmol) in anhydrous THF (40.0 mL) at −78 °C was added *n*-BuLi (1.6 M, 11.5 mL, 18.3 mmol) dropwise under argon atmosphere. The mixture was stirred for an hour at −78 °C and diethyl clorophosphate (0.67 mL, 4.6 mmol) was added slowly. The reaction mixture was stirred at 0 °C for an hour. The reaction was stopped by adding satd NH_4Cl solution (10 mL) followed by extraction with dichloromethane (3×50 mL). The combined organic layers were dried over anhyd Na_2SO_4. The desiccant was filtered off and the filtrate was concentrated. The residue was purified by flash column chromatography (50–80% EtOAc in hexanes) to afford unreacted starting material (450 mg) and product **9** as colourless oil (800 mg, 65% based on recovered starting material). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.36 (td, J = 7.2, 0.9 Hz, 6H, OCH_2Me), 2.37 (d, J = 5.4 Hz, 3H, NMe), 3.60 (d, J = 16.2 Hz, 2H, SCH_2P), 4.22 (qt, J = 6.9 Hz, 0.9 Hz, 4H, OCH_2Me), 5.26 (q, J = 5.1 Hz, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 16.14 (d, J = 6 Hz, OCH_2CH_3), 29.59 (NCH_3), 46.90 (d, J = 138.5 Hz, PCH_2S), 63.53 (d, J = 6.1 Hz, OCH_2CH_3). ^{31}P NMR (121 MHz, $CDCl_3$) δ ppm 13.52. ESI-HRMS for $[C_6H_{16}NO_5PS+H]^+$ Calcd, 246.0565. Found, 246.0548.

3.0.2. 1-((2*R*,4*S*,5*R*)-4-((*tert*-Butyldimethylsilyl)oxy)-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (12)

Compound **11** (9.71 g, 20.64 mmol) and anhydrous THF (106.4 mL) was placed in a Teflon flask under inert condition and cooled to 0 °C. In a separate polypropylene flask, anhydrous THF (79.8 mL) and anhydrous pyridine (31.9 mL) were placed under inert atomsphere; to this at 0 °C ~70%HF in pyridine (28.7 mL) was added drop wise. The chilled THF-Py–HF.Py mixture was added dropwise to a flask containing compound **11** also at 0 °C and stirred at this temperature for 1 h. The cold bath was removed and reaction continued at room temperature for 45 min. The reaction mixture was poured to an ice-cold solution of $NaHCO_3$ (100 g in 500 mL) with vigorous stirring. The compound was extracted in ethyl acetate (3×150 mL), washed with brine and dried over anhyd Na_2SO_4. The residue after evaporation was subjected to flash column chromatography (20–50% EtOAc in hexanes) to obtain compound **12** as white foam (2.615 g, 36%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.07 (s, 6H, $SiMe_2$), 0.88 (s, 9H, *t*Bu), 1.88 (s, 3H, 5-Me), 2.20 (ddd, J = 13.25, 6.37, 3.81 Hz, 1H, 2′-H), 2.32 (dt, J = 13.40, 6.63 Hz, 1H, 2′-H), 2.98–3.13 (m, 1H, 5′-OH), 3.68–3.80 (m, 1H, 5′-H), 3.85–3.96 (m, 2H, 5′ & 4′-H), 4.48 (dt, J = 6.44, 3.51 Hz, 1H, 3′-H), 6.16 (t, J = 6.74 Hz, 1H, 1′-H), 7.35–7.48 (m, 1H, 6-H), 9.31 (br s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm −4.66 (SiMe-C), −4.50 (SiMe-C), 12.69 (5-Me-C), 18.15 (*t*Bu-tC), 25.92 (*t*Bu-C), 40.76 (2′-C), 62.11 (5′-C), 71.81 (3′-C), 86.87 (1′-C), 87.84 (4′-C), 111.13 (5-C), 137.25 (6-C), 150.67 (2-C), 164.26 (4-C). ESI-HRMS for $[C_{16}H_{28}N_2O_5Si+H]^+$ Calcd, 357.1846. Found, 357.1847.

3.0.3. 1-((2*R*,4*S*,5*R*)-4-((*tert*-Butyldimethylsilyl)oxy)-5-((*E*)-2-(methylsulfonyl)vinyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (14)

To a solution of Dess–Martin reagent (1.28 g, 3 mmol) in anhydrous dichloromethane (5 mL) at 0 °C under argon atmosphere was added compound **12** (713 mg, 2 mmol) in Dichloromethane (5 mL) and the reaction continued at room temperature. After 4 h saturated $NaHCO_3$ solution (10 mL) was added and the product extracted in dichloromethane (3×20 mL). The organic layers were combined, washed with brine, dried over anhyd Na_2SO_4, filtered

The filtrate was evaporated and dried under high vacuum to give aldehyde **13** as white foamy residue which was used without purification for Horner–Wittig reaction.

In a separate flask, diethyl((methylsulfonyl)methyl)phosphonate **6** (553 mg, 2.4 mmol) was dissolved in anhydrous THF (20 mL) under argon atmosphere. The mixture was brought to −78 °C and *n*-BuLi (1.6 M in hexanes, 1.38 mL, 2.2 mmol) was added slowly and stirred for 15 min. The solution of the above aldehyde **13** (2 mmol) in anhydrous THF (15 mL) was added dropwise. The flask was kept at −78 °C for 1 h and at room temperature overnight (18 h). Water (10 mL) was added and the product extracted in ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine, dried over anhyd Na_2SO_4, filtered and filtrate was evaporated. The residue was purified by flash column chromatography (20–50% EtOAc in hexanes) to afford **14** as white solid (175 mg, 20%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.03 (s, 3H, SiMe), 0.04 (s, 3H, SiMe), 0.84 (s, 9H, *t*-Bu), 1.89 (d, *J* = 1.17 Hz, 3H, 5-Me), 2.16–2.24 (m, 2H, 2′-H), 2.91 (s, 3H, SO_2Me), 4.25 (dt, *J* = 6.37, 4.87 Hz, 1H, 3′-H), 4.33–4.43 (m, 1H, 4′-H), 6.22 (t, *J* = 6.74 Hz, 1H, 1′-H) 6.62 (dd, *J* = 15.08, 1.90 Hz, 1H, 6′-H), 6.93 (dd, *J* = 14.94, 4.10 Hz, 1H, 5′-H), 6.96 (d, *J* = 1.17 Hz, 1H, 6-H), 8.39 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm -4.78 (SiMe-C), −4.66 (SiMe-C), 12.64 (5Me-C), 17.90 (*t*Bu-tC), 25.63 (*t*BuMe-C), 39.46 (2′-C), 42.68 (SO_2Me–C), 74.66 (3′-C), 84.05 (4′-C), 85.56 (1′-C), 111.93 (5-C), 130.75 (6′-C), 135.28 (6-C), 142.90 (5′-C), 150.00 (2-C), 163.14 (4-C). ESI-HRMS for $[C_{18}H_{30}N_2O_6SSi+H]^+$ Calcd, 431.1672. Found, 431.1519.

3.0.4. 1-((2*R*,4*S*,5*R*)-4-((*tert*-Butyldimethylsilyl)oxy)-5-(2-(methylsulfonyl)ethyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (15)

To a solution of **14** (175 mg, 0.41 mmol) in ethyl acetate (10.0 mL) was added Pd-C (10% Pd, ∼50% H_2O, 100 mg) and stirred with purging H_2 gas through the reaction mixture at room temperature for 4 h. The catalyst was filtered over celite and filtrate was evaporated. The residue was dried under high vacuum to afford practically pure compound **15** (175 mg) which was used as such for the next step. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.02 (s, 3H, SiMe), 0.03 (s, 3H, SiMe), 0.83 (s, 9H, *t*Bu), 1.89 (d, *J* = 1.17 Hz, 3H, 5-Me), 1.92–2.07 (m, 2H, 5′-H), 2.14–2.30 (m, 2H, 2′-H), 2.88 (s, 3H, SO_2Me), 3.04 (ddd, *J* = 13.7, 11.1, 5.7 Hz, 1H, 6′-H), 3.13–3.25 (m, 1H, 6′-H), 3.75 (dt, *J* = 10.40, 3.88 Hz, 1H, 4′-H), 4.08–4.12 (m, 1H, 3′-H), 6.09 (t, *J* = 6.88 Hz, 1H, 1′-H), 6.99 (d, *J* = 1.46 Hz, 1H, 6-H), 8.08 (br s, 1H, NH). ESI-HRMS for $[C_{18}H_{32}N_2O_6SSi+H]^+$ Calcd, 433.1829. Found, 433.0574.

3.0.5. 1-((2*R*,4*S*,5*R*)-4-hydroxy-5-(2-(methylsulfonyl)ethyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (16)

To a solution of compound **15** (175 mg, 0.41 mmol) in anhydrous THF (3.0 mL) was added tetrabutylammonium fluoride (TBAF, 0.14 mL, 0.49 mmol) dropwise. The flask was heated to 40 °C overnight. The volatile materials were evaporated under high vacuum and the residue was purified by flash column chromatography (3% MeOH in CH_2Cl_2). The product was purified further by crystallization/triturating in methanol to afford compound **16** as a colorless needles (25 mg, 20%). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.80 (d, *J* = 1.17 Hz, 3H, 5-Me), 1.87–2.00 (m, 1H, 5′-H), 2.00–2.15 (m, 2H, 2′ & 5′-H), 2.25 (dt, *J* = 13.84, 6.99 Hz, 1H, 2′-H), 3.00 (s, 3H, SO_2Me), 3.19 (t, *J* = 8.05 Hz, 2H, 6′-H), 3.72 (dt, *J* = 8.57, 4.36 Hz, 1H, 4′-H), 4.08–4.18 (m, 1H, 3′-H), 5.34 (d, *J* = 4.39 Hz, 1H, 3′-OH), 6.15 (t, *J* = 7.03 Hz, 1H, 1′-H), 7.44 (d, *J* = 1.17 Hz, 1H, 6-H), 11.30 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 12.06 (5-Me-C), 25.78 (5′-C), 38.05 (2′-C), 40.15 (SO_2Me-C), 50.39 (6′-C), 72.84 (3′-C), 83.46 (1′-C), 83.83 (4′-C), 109.97 (5-C), 136.17 (6-C), 150.47 (2-C), 163.68 (4-C). ESI-HRMS for $[C_{12}H_{18}N_2O_6S+H]^+$ Calcd, 319.0964. Found 319.0963.

3.0.6. (*E*)-2-((2*R*,3*S*,5*R*)-3-((*tert*-Butyldimethylsilyl)oxy)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-2-yl)-*N*-methylethenesulfonamide (17)

Compound **12** (100 mg, 0.28 mmol) was reacted in similar fashion as described for **14** to get the aldehyde **13**. In a separate flask, to the solution of diethyl ((N-methylsulfamoyl)methyl)phosphonate **11** (83 mg, 0.36 mmol) in anhydrous THF (3 mL) at −78 °C under inert atmosphere was added *n*-BuLi (1.6 M in hexanes, 0.39 mL, 0.62 mmol) drop wise and stirred for 1 h. A solution of above aldehyde 13 (0.28 mmol) in THF (3 mL) was added and the mixture was kept at −78 °C for 1 h then allowed to stir at room temperature overnight (18 h). Water (5 mL) was added and the reaction mixture was extracted with ethyl acetate (3 × 25 mL). The combined organic layer was washed with brine, dried over anhyd Na_2SO_4, filtered, and evaporated. The residue was purified by preperative TLC (50% EtOAc in toluene) to obtain 17 as a white foam (60 mg, 48%). ^{1}H NMR (300 MHz, CDCl3): δ 0.10 (s, 3H, SiMe), 0.11 (s, 3H, SiMe), 0.91 (s, 9H, *t*-BuSi), 1.96 (d, *J* = 1.2 Hz, 3H, 5-Me), 2.23–2.29 (m, 2H, 2′-H), 2.76 (s, 3H, NHMe), 4.28–4.33 (m, 1H, 4′-H), 4.41 (td, *J* = 4.8, 1.2 Hz, 1H, 3′-H), 6.30 (t, *J* = 6.9 Hz, 1H, 1′H), 6.47 (dd, *J* = 15.3, 1.5 Hz, 1H, 6′-H), 6.82 (dd, *J* = 15.3, 4.5 Hz, 1H, 5′-H), 7.04 (d, *J* = 1.2 Hz, 1H, 6-H) ESI-HRMS for [C18H31N3O6SSi+H]$^+$ Calcd, 446.1781. Found, 446.1773.

3.0.7. 2-((2*R*,3*S*,5*R*)-3-((*tert*-Butyldimethylsilyl)oxy)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-2-yl)-*N*-methylethanesulfonamide (18)

To a solution of **17** (60 mg, 0.135 mmol) in 5:1 MeOH-THF (1.5 mL) at 0 °C was added $NiCl.6H_2O$ (16 mg, 0.075 mmol) and $NaBH_4$ (10.2 mg, 0.27 mmol). The reaction mixture was stirred for 1 h at 0 °C and the solvent was evaporated. The residue suspended in ethyl acetate, filtrated over celite, the filtrate was evaporated. The residue obtained was dissolved in THF (1.5 mL) and was added TBAF (80 μL, 0.268 mmol). The reaction mixture was stirred at 40 °C for 1 h. The solvent was evaporated to dryness and the residue was purified by flash column chromatography (3–7% MeOH in CH_2Cl_2) to afford **18** (5 mg, 12%) as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.80 (d, *J* = 0.88 Hz, 3H, 5-Me), 1.91 (td, *J* = 9.15, 4.54 Hz, 1H, 5′-H), 1.96–2.10 (m, 2H, 2′ & 5′-H), 2.24 (dt, *J* = 13.84, 6.99 Hz, 1H, 2′-H), 2.57 (d, *J* = 4.98 Hz, 3H, N-Me), 3.07 (dt, *J* = 9.81, 5.64 Hz, 2H, 6′-H), 3.73 (dt, *J* = 8.42, 4.43 Hz, 1H, 4′-H), 4.11 (dd, *J* = 6.74, 4.10 Hz, 1H, 3′-H), 5.34 (d, *J* = 4.39 Hz, 1H, 3′-OH), 6.14 (t, *J* = 6.88 Hz, 1H, 1′-H), 6.92 (q, *J* = 4.98 Hz, 1H, MeNH) 7.42 (d, *J* = 1.17 Hz, 1H, 6-H), 11.30 (br s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 11.96 (5-Me-C), 27.01 (5′-C), 28.47 (NMe-C), 38.04 (2′-C), 46.01 (6′-C), 72.73 (3′-C), 83.29 (1′-C), 83.62 (4′-C), 109.84 (5-C), 136.00 (6-C), 150.33 (2-C), 163. 55 (4-C). ESI-HRMS for $[C_{12}H_{19}N_3O_6S+H]^+$ Calcd, 334.1073. Found, 334.1085.

3.0.8. (*E*)-3-((2*R*,3*S*,5*R*)-3-((*tert*-Butyldimethylsilyl)oxy)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-2-yl)acrylonitrile (19) [19]

Compound **12** (1.22 g, 3.43 mmol) was reacted in similar fashion as described for **14** to get the aldehyde **13**. In a separate flask, to a solution of cyanomethyltriphenylphosphonium chloride (3.48 g, 10.3 mmol) in anhydrous THF (35 mL) at 0 °C under argon atmosphere was added drop wise *n*-BuLi (1.6 M in hexanes, 6.4 mL, 10.3 mmol) and stirred for 30 min. To this ylide at 0 °C was added slowly a solution of above aldehyde **13** (3.43 mmol) in anhydrous THF (15 mL) and stirred at room temperature overnight. The

reaction was quenched with water (30 mL) and extracted with ethyl acetate (3 × 100 mL). Combined organic layer was washed with brine, dried over anhyd Na_2SO_4, filtered and evaporated. The residue obtained was purified by flash column chromatography (15–30% EtOAc in hexanes) to afford **19** as a light yellow foam (740.4 mg, 57%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.09 (s, 3H, SiMe), 0.10 (s, 3H, SiMe), 0.90 (s, 9H, *t*Bu), 1.96 (d, J = 1.17 Hz, 3H, 5-Me), 2.27–2.36 (m, 2H, 2′-H), 4.27–4.35 (m, 2H, 3′-H and 4′-H), 5.67 (dd, J = 16.26, 1.61 Hz, 1H, 6′-H), 6.19 (t, J = 6.59 Hz, 1H, 1′-H), 6.80 (dd, J = 16.11, 4.69 Hz, 1H, 5′-H), 7.01 (d, J = 1.17 Hz, 1H, 6-H), 9.46 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm −4.82 (SiMe-C), −4.63 (SiMe-C), 12.65 (5-Me-C), 17.89 (*t*Bu-tC), 25.63 (*t*Bu-C), 39.79 (2′-C) 74.69 (3′-C), 84.96 (4′-C), 86.14 (1′-C), 101.00 (6′-C), 111.74 (5-C), 116.56 (7′-C), 135.74 (6-C) 149.95 (5′-C), 150.25 (2-C), 163.80 (4-C). ESI-HRMS for $[C_{18}H_{27}N_3O_4Si+H]^+$ Calcd, 378.1849. Found, 378.1852.

3.0.9. 3-((2*R*,3*S*,5*R*)-3-((*tert*-Butyldimethylsilyl)oxy)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-2-yl)propanenitrile (20)

To a solution of **19** (443 mg, 1.18 mmol) in 3:1 anhydrous pyridine–MeOH (2 mL) was added $NaBH_4$ (45 mg, 1.18 mmol) and the reaction mixture was stirred at 120 °C under inert atmosphere for 4 h. The reaction mixture was evaporated and the residue was purified by flash column chromatography (30–50% EtOAc in hexanes) to afford **20** as white foam (240 mg, 54%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.09 (s, 3H, SiMe), 0.10 (s, 3H, SiMe), 0.90 (s, 9H, *t*-Bu), 1.84–1.93 (m, 1H, 5′-H), 1.95 (d, J = 1.46 Hz, 3H, 5-Me), 2.02–2.16 (m, 1H, 5′-H), 2.27 (t, J = 6.44 Hz, 2H, 2′-H), 2.41–2.62 (m, 2H, 6′-H), 3.80 (ddd, J = 9.30, 5.35, 3.81 Hz, 1H, 4′-H), 4.12–4.20 (m, 1H, 3′-H), 6.08 (t, J = 6.59 Hz, 1H, 1′-H), 7.05 (d, J = 1.17 Hz, 1H, 6-H), 8.44 (br s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm −4.81 (SiMe-C), −4.53 (SiMe-C), 12.62 (5-Me-C), 14.22 (6′-C), 17.91 (*t*Bu-tC), 25.68 (*t*Bu-C), 28.94 (5′-C), 40.15 (2′-C), 74.43 (3′-C), 84.04 (4′-C), 85.60 (1′-C), 111.35 (5-C), 119.11 (7′-C), 135.87 (6-C), 149.86 (2-C), 163.32 (6-C). ESI-HRMS for $[C_{18}H_{29}N_3O_4Si+H]^+$ Calcd, 380.2006. Found, 380.2015.

3.0.10. 3-((2*R*,3*S*,5*R*)-3-hydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-2-yl)propanenitrile (21)

To the stirring solution of compound **20** (100 mg, 0.26 mmol) in anhydrous THF (2 mL) was added tetrabutylammonium fluoride (TBAF, 1 M in THF, 0.5 mL, 0.5 mmol) under inert atmosphere and stirred at room temperature for 4 h. The reaction mixture was evaporated under vacuum and the residue was purified by flash column chromatography (EtOAc) to afford product **21** as a white solid (53 mg, 76%). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.80 (d, J = 1.17 Hz, 3H, 5-Me), 1.83–1.98 (m, 2H, 5′-H), 2.05 (ddd, J = 13.55, 6.52, 3.95 Hz, 1H, 2′-H), 2.22 (dt, J = 13.77, 6.88 Hz, 1H, 2′-H), 2.52–2.62 (m, 2H, 6′-H), 3.63–3.74 (m, 1H, 4′-H), 4.04–4.17 (m, 1H, 5′-H), 5.34 (d, J = 4.39 Hz, 1H, 3′-OH), 6.15 (t, J = 6.88 Hz, 1H, 1′-H), 7.44 (d, J = 1.17 Hz, 1H, 6-H), 11.30 (br s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 11.96 (5-Me-C), 13.20 (6′-C), 28.37 (5′-C),v 38.01 (2′-C), 72.50 (3′-C), 83.42 (1′-C), 83.92 (4′-C), 109.75 (5-C), 120.30 (7′-C), 136.13 (6-C), 150.33 (2-C), 163.56 (4-C). ESI-HRMS for $[C_{12}H_{15}N_3O_4-H]^-$ Calcd, 264.099. Found, 264.0617.

3.0.11. 1-((2*R*,4*S*,5*R*)-5-(2-(1*H*-tetrazol-5-yl)ethyl)-4-((*tert*-Butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (22)

Compound **20** (120 mg, 0.32 mmol) was dessolved in anhydrous toluene (5 mL), to this was added dibutyltin oxide (Bu_2SnO, 16 mg) and azidotrimethylsilane ($TMSN_3$, 158 μL, 1.6 mmol) under argon atmosphere. The reaction vessel was sealed with septum and stirred at 110 °C for 4 h. The volatiles were evaporated under reduced pressure and the residue was purified by flash column chromatography (2% AcOH/ EtOAc) to afford **22** as a white foam (43 mg, 32%). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.00 (s, 6H, $SiMe_2$), 0.78 (s, 9H, *t*-Bu), 1.73 (d, J = 0.88 Hz, 3H, 5-Me), 1.86–2.07 (m, 3H, 2′-H and 5′-H), 2.21 (dt, J = 13.62, 6.96 Hz, 1H, 2′-H), 2.80–2.96 (m, 2H, 6′-H), 3.61 (dt, J = 8.42, 4.43 Hz, 1H, 4′-H), 4.20 (dt, J = 6.59, 4.03 Hz, 1H, 3′-H), 6.04 (t, J = 6.88 Hz, 1H, 1′-H), 7.33 (d, J = 1.17 Hz, 1H, 6-H), 11.23 (br s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm −4.89 (SiMe-C), −4.72 (SiMe-C), 12.10 (5-Me-C), 17.61 (*t*Bu-tC), 19.63 (6′-C), 25.65 (*t*Bu-C), 30.42 (5′-C), 38.60 (2′-C), 74.35 (3′-C), 83.46 (1′-C), 84.44 (4′-C), 109.89 (5-C), 136.21 (6-C), 150.39 (2-C), 155.69 (7′-C) 163.66 (4-C). ESI-HRMS for $[C_{18}H_{30}N_6O_4Si-H]^-$ Calcd, 421.2020; Found, 421.1249.

3.0.12. 1-((2*R*,4*S*,5*R*)-5-(2-(1*H*-tetrazol-5-yl)ethyl)-4-hydroxytetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (23)

Compound **22** (40 mg, 0.1 mmol) was dissolved in anhydrous THF (2 mL) and added TBAF (0.5 mL, 0.5 mmol) at room temperature. After 4 h, methanol (2 mL) was added, followed by $CaCO_3$ (1 g) with vigorous stirring. After 10 min was added Dowex resin (H^+ form, 1 g) portion wise. The mixture was stirred for 30 min and filtered over a pad of celite. The filtrate was concentrated and purified by flash column chromatography (5–8% MeOH + 2% AcOH in CH_2Cl_2) to afford title compound **23** as a white solid (10 mg, 34%). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.80 (d, J = 1.17 Hz, 3H, 5-Me), 1.89–2.14 (m, 3H, 2′-H and 5′-H), 2.14–2.33 (m, 1H, 2′-H), 2.87–3.06 (m, 2H, 6′-H), 3.66 (dt, J = 8.57, 4.36 Hz, 1H, 4′-H), 4.05–4.17 (m, 1H, 3′-H), 5.30 (br s, 1H, 3′-OH), 6.14 (t, J = 6.88 Hz, 1H, 1′-H), 7.39 (d, J = 0.88 Hz, 1H, 6-H), 11.30 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 11.99 (5-Me-C), 19.39 (6′-C), 30.56 (5′-C), 38.20 (2′-C), 72.73 (3′-C), 83.18 (1′-C), 84.30 (4′-C), 109.79 (5-C), 135.94 (6-C), 150.33 (2-C), 155.07 (7′-C), 163.56 (4-C). ESI-HRMS for $[C_{12}H_{16}N_6O_4-H]^-$ Calcd, 307.1155. Found, 307.0597.

3.0.13. 1-((2*R*,4*S*,5*R*)-4-((*tert*-Butyldimethylsilyl)oxy)-5-(hydroxymethyl)tetrahydrofuran-2-yl)pyrimidine-2,4(1*H*,3*H*)-dione (26)

Removal of primary TBS group using the procedure described for compound **12**, compound **25** (1.8 g, 4 mmol) rendered **26** (600 mg, 53%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.09 (s, 6H, SiMe), 0.90 (s, 9H, *t*Bu), 2.24–2.32 (m, 2H, 2′-H) 2.50 (br s, 1H, 5′-OH), 3.69–3.82 (m, 1H, 5′-H), 3.87–4.00 (m, 2H, 4′-H and 5′-H), 4.43–4.55 (m, 1H, 3′-H), 5.74 (dd, J = 8.20, 2.05 Hz, 1H, 5-H), 6.18 (t, J = 6.59 Hz, 1H, 1′-H), 7.65 (d, J = 8.20 Hz, 1H, 6-H), 8.89 (br s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm −4.64 (SiMe-C), −4.47 (SiMe-C), 18.18 (*t*Bu-tC), 25.93 (*t*Bu-C), 41.09 (2′-C), 62.09 (5′-C), 71.64 (3′-C), 87.02 (1′-C), 87.82 (4′-C), 102.71 (5-C), 141.31 (6-C), 150.42 (2-C), 163.40 (4-C). ESI-HRMS for $[C_{15}H_{26}N_2O_5Si+H]^+$ Calcd, 343.1689. Found, 343.1682.

3.0.14. (*E*)-3-((2*R*,3*S*,5*R*)-3-((*tert*-Butyldimethylsilyl)oxy)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-2-yl)acrylonitrile (27)

Following the oxidation and Wittig reaction procedure described for compound **19**, compound **26** (600 mg, 1.75 mmol) rendered **27** (325 mg, 51%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.10 (s, 6H, $SiMe_2$), 0.90 (s, 9H, *t*Bu), 2.19–2.43 (m, 2H, 2′-H), 4.24–4.30 (m, 1H, 3′-H), 4.30–4.37 (m, 1H, 4′-H), 5.67 (dd, J = 16.40, 1.76 Hz, 1H, 6′-H), 5.81 (dd, J = 8.20, 2.34 Hz, 1H, 5-H), 6.19 (t,

J = 6.44 Hz, 1H, 1′-H), 6.77 (dd, *J* = 16.11, 4.69 Hz, 1H, 5′-H), 7.21 (d, *J* = 8.20 Hz, 1H, 6-H), 8.65 (br s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm −4.82 (SiMe-C), −4.63 (SiMe-C), 17.90 (*t*Bu-tC), 25.63 (*t*Bu-C), 40.06 (2′-C), 74.59 (3′-C), 85.04 (4′-C), 86.30 (1′-C), 101.19 (6′-C), 103.21 (5-C), 116.42 (7′-C), 139.83 (6-C), 149.61 (5′-C), 149.82 (2-C), 162.71 (4-C). ESI-HRMS for $[C_{17}H_{25}N_3O_4Si+CH_3CN+H]^+$ Calcd, 405.1958. Found, 405.1955.

3.0.15. 3-((2*R*,3*S*,5*R*)-3-((*tert*-Butyldimethylsilyl)oxy)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-2-yl)propanenitrile (28)

Following the reaction procedure described for compound **15** but using methanol as solvent, compound **27** (55 mg, 0.15 mmol) gave **28** as a white solid (50 mg, 89%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.09 (d, 6H, $SiMe_2$), 0.90 (s, 9H, *t*Bu), 1.89 (dddd, *J* = 13.84, 9.45, 7.47, 6.15 Hz, 1H, 5′-H), 2.03–2.15 (m, 1H, 5′-H), 2.19–2.38 (m, 2H, 2′-H), 2.42–2.62 (m, 2H, 6′-H), 3.82 (ddd, *J* = 9.37, 5.42, 3.66 Hz, 1H, 4′-H), 4.1 δ 4 (dt, *J* = 7.03, 5.42 Hz, 1H, 3′-H), 5.77 (d, *J* = 7.91 Hz, 1H, 5-H), 6.08 (t, *J* = 6.59 Hz, 1H, 1′-H), 7.26 (d, *J* = 8.20 Hz, 1H, 6-H), 8.40 (br s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm −4.83 (SiMe-C), −4.54 (SiMe-C), 14.25 (6′-C), 17.90 (*t*Bu-tC), 25.67 (*t*Bu-C), 28.98 (5′-C), 40.40 (2′-C), 74.37 (3′-C), 84.20 (4′-C), 85.95 (1′-C), 102.83 (5-C), 119.01 (7′-C), 140.08 (6-C), 149.84 (2-C), 162.84 (4-C). ESI-HRMS for $[C_{17}H_{27}N_3O_4Si+H]^+$ Calcd, 366.1849. Found, 366.1842.

3.0.16. 3-((2*R*,3*S*,5*R*)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3-hydroxytetrahydrofuran-2-yl)propanenitrile (29)

Following the reaction procedure described for compound **21**, compound **28** (50 mg, 0.14 mmol) rendered **29** (25 mg, 73%) as a white solid. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 1.89–2.14 (m, 2H, 5′-H), 2.20–2.37 (m, 2H, 2′-H), 2.48–2.69 (m, 2H, 6′-H), 3.85 (dt, *J* = 9.23, 4.47 Hz, 1H, 4′-H), 4.13–4.25 (m, 1H, 3′-H), 5.71 (d, *J* = 7.91 Hz, 1H, 5-H), 6.18 (t, *J* = 6.74 Hz, 1H, 1′-H), 7.62 (d, *J* = 8.20 Hz, 1H, 6-H). ^{13}C NMR (75 MHz, CD_3OD) δ ppm 14.48 (6′-C), 30.32 (5′-C), 40.21 (2′-C), 74.68 (3′-C), 85.87 (4′-C), 86.71 (1′-C), 103.04 (5-C), 120.79 (7′-C), 142.57 (6-C), 152.07 (2-C), 166.15 (4-C). ESI-HRMS for $[C_{11}H_{13}N_3O_4-H]^-$ Calcd, 250.0828. Found, 250.0833.

3.0.17. (1-((2*R*,4*S*,5*R*)-4-((*tert*-Butyldimethylsilyl)oxy)-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)methyl acetate (32)

Following the procedure described for the synthesis of **12**, compound **31** (1 g, 1.94 mmol) rendered **32** (265 mg, 33%) as a white foam.

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.09 (s, 6H, SiMe), 0.90 (s, 9H, *t*Bu), 2.06 (s, 3H, OAc), 2.18–2.40 (m, 2H, 2′-H), 3.77 (dd, *J* = 12.7, 3.2 Hz,1H, 5′-H), 3.88–4.04 (m, 2H, 5′ & 4′-H), 4.52 (dt, *J* = 5.80, 3.70 Hz, 1H, 3′-H), 4.79–4.97 (m, 2H, 5-CH_2O), 6.24 (t, *J* = 6.49 Hz, 1H, 1′-H), 8.11 (s, 1H, 6-H), 9.02 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm −4.90, −4.74 (SiMe-C), 17.94 (*t*Bu-tC), 21.14 (Ac-C), 25.69 (*t*Bu-C), 41.36 (1′-C), 58.82 (5-CH_2O–C), 61.82 (5′-C), 71.66 (3′-C), 86.49 (1′-C), 88.12 (4′-C), 109.02 (5-C), 142.86 (6-C), 149.94 (2-C), 162.58 (4-C), 171.96 (Ac-C= O). ESI-HRMS for $[C_{18}H_{30}N_2O_7Si+H]^+$ Calcd, 415.1901. Found, 415.1904.

3.0.18. 5-(((Benzyloxy)methoxy)methyl)-3-((benzyloxy)methyl)-1-((2*R*,4*S*,5*R*)-4-((*tert*-butyldimethylsilyl)oxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-2-yl)pyrimidine-2,4(1*H*,3*H*)-dione (34)

To a mixture of compound **33** (150 mg, 0.31 mmol) in anhydrous DMF (2.5 mL) and anhydrous diisopropylethylamine (DIPEA, 160 μL, 0.92 mmol) at 0 °C under inert atmosphere was added drop wise benzyloxymethyl chloride (~75% BOMCl, 94 μL, 0.68 mmol) and stirred at room temperature overnight. Saturated aq.NH_4Cl (5 mL) was added to quench the reaction. The products were extracted in ethyl acetate (50 mL), organic layer washed with water, brine, dried over anhyd Na_2SO_4 and evaporated. The residue was purified by flash column chromatography (5–10% EtOAc in hexanes) to yield **34** (80 mg, 36%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.08–0.12 (m, 12H, SiMe), 0.88–0.94 (m, 18H, *t*Bu), 1.97 (ddd, *J* = 13.34, 7.64, 5.97 Hz, 1H, 2′-H), 2.30 (ddd, *J* = 13.16, 5.74, 2.44 Hz, 1H, 2′-H), 3.76 (dd, *J* = 11.40, 3.08 Hz, 1H, 5′-H), 3.82 (dd, *J* = 11.22, 3.26 Hz, 1H, 5′-H), 3.96 (q, *J* = 3.08 Hz, 1H, 4′-H), 4.34–4.47 (m, 3H, 3′-H & 5-CH_2), 4.65 (s, 2H, OCH_2Ph), 4.71 (s, 2H, OCH_2Ph), 4.80–4.87 (m, 2H, OCH_2O), 5.50 (s, 2H, NCH_2O), 6.31 (dd, *J* = 7.87, 5.70 Hz, 1H, 1′-H), 7.22–7.41 (m, 10H, Ar), 7.65 (s, 1H, 6-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm −5.50, −5.40, −4.84, −4.65 (SiMe-C), 17.98, 18.39 (*t*Bu-tC), 25.73, 25.93 (*t*Bu-C), 41.35 (1′-C), 62.99 (5-CH_2-C), 63.07 (5′-C), 69.39 (Bn-C), 70.45 (NCH_2O–C), 72.24 (3′-C), 72.28 (Bn-C), 85.95 (1′-C), 87.94 (4′-C), 94.36 (OCH_2O–C), 110.72 (5-C) 126.97, 127.61, 127.69, 127.92, 128.26, 128.39, 128.56 (Ar-C), 136.92 (6-C), 137.70, 137.93 (Ar-C), 150.80 (2-C), 162.24 (4-C). ESI-HRMS for $[C_{38}H_{58}N_2O_8Si_2+H]^+$ Calcd, 727.381. Found, 727.3805.

3.0.19. 1-((2*R*,4*S*,5*R*)-4-((*tert*-Butyldimethylsilyl)oxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-2-yl)-5-(((2-(trimethylsilyl)ethoxy)methoxy)methyl)-3-((2-(trimethylsilyl)ethoxy)methyl)pyrimidine-2,4(1*H*,3*H*)-dione (35)

Under an inert condition compound **33** (486 mg, 1 mmol) was dissolved in anhydrous CH_2Cl_2 (3 mL) and anhydrous DIPEA (0.5 mL, 3 mmol) and added ethyltrimethysilylchloromethyl ether (SEMCl, 0.44 ml, 2.5 mmol). The reaction was continued at 40 °C for 6 h. The reaction mixture was partitioned between brine (5 mL) and ethyl acetate (20 mL). The organic layer was separated and dried over anhyd Na_2SO_4. The residue after evaporation of organic phase was purified by flash column chromatography (5% EtOAc in hexanes) to afford compound **35** (370 mg, 50%) as a glass. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.00 (s, 9H, $SiMe_3$-SEM), 0.03 (s, 9H, $SiMe_3$-SEM), 0.08 (s, 3H, SiMe-TBS), 0.09 (s, 3H, SiMe-TBS), 0.11 (s, 6H, $SiMe_2$-TBS), 0.90 (s, 9H, *t*Bu), 0.92 (s, 9H, *t*Bu), 0.94–1.02 (m, 4H, Me_3SiCH_2), 2.00 (ddd, *J* = 15.3 Hz,7.8 Hz, 1.8 Hz, 1H, 2′-H), 2.31 (ddd, *J* = 13.2 Hz, 5.7 Hz, 2.4 Hz, 1H, 2′-H), 3.60–3.73 (m, 4H, $OCH_2CH_2SiMe_3$), 3.76 (dd, *J* = 11.10, 3.15 Hz, 1H, 5′-H), 3.82 (dd, *J* = 11.29, 3.33 Hz, 1H, 5′-H), 3.96 (q, *J* = 2.96 Hz, 1H, 4′-H), 4.31 (d, *J* = 12.24 Hz, 1H, 5-CH_2), 4.37 (d, *J* = 12.0 Hz, 1H, 5-CH_2), 4.37–4.42 (m, 1H, 3′-H), 4.74 (s, 2H, OCH_2O), 5.40 (s, 2H, NCH_2O), 6.32 (1H, dd, *J* = 7.8 Hz, 5.7 Hz, 1′-H), 7.65 (s, 1H, 6-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm -5.50, -5.38, -4.84, -4.67 (TBSSiMe–C), −1.44, −1.38 (SEMSiMe_3–C), 17.97 (*t*-Bu-tC), 18.07, 18.11 (SEMS-iCH_2–C); 18.40 (t-Bu-tC), 25.72, 25.95 (*t*Bu-C), 41.34 (2′-C), 62.96 (5-CH_2O–C), 62.99 (5′-C), 65.28, 67.49 (SEMCH_2O–C), 70.22 (NCH_2O–C), 72.43 (3′-C), 86.11 (1′-C), 88.06 (4′-C), 94.94 (OCH_2O–C), 111.08 (5-C), 136.71 (6-C), 150.99 (2-C), 162.34 (4-C). ESI-HRMS for $[C_{34}H_{70}N_2O_8Si_4+H]^+$ Calcd, 747.4287. Found, 747.4144.

3.0.20. 1-((2*R*,4*S*,5*R*)-4-((*tert*-Butyldimethylsilyl)oxy)-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-(((2-(trimethylsilyl)ethoxy)methoxy)methyl)-3-((2-(trimethylsilyl)ethoxy)methyl)pyrimidine-2,4(1*H*,3*H*)-dione (36)

Following the procedure described for the synthesis of **12**, compound **35** (350 mg, 0.48 mmol) gave compound **36** as a glass

(114 mg, 38%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.00 (s, 9H, $SiMe_3$-SEM), 0.03 (s, 9H, $SiMe_3$-SEM), 0.09 (s, 6H, SiMe-TBS), 0.90 (s, 9H, *t*Bu); 0.92–1.00 (m, 4H, SEMSiCH_2), 2.22–2.41 (m, 2H, 2′-H), 2.58 (t, *J* = 4.8 Hz, 1H, 5′-OH), 3.60–3.72 (m, 4H, SEMCH_2O), 3.72–3.80 (m, 1H, 5′-H), 3.87–3.93 (m, 1H, 5′-H), 3.93–3.98 (m, 1H, 4′-H), 4.39 (s, 2H, 5-CH_2O), 4.51 (1H, dt, *J* = 6.6, 3.81 Hz, 3′-H), 4.73 (s, 2H, OCH_2O), 5.39 (s, 2H, NCH_2O), 6.20 (t, *J* = 6.44 Hz, 1H, 1′-H), 7.75 (s, 1H, 6-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm −4.86, −4.69 (TBSSiMe–C), −1.43, −1.42 (SEMSiMe_3–C), 17.95 (*t*Bu-tC), 18.12 (SEMSiCH_2–C), 25.71 (*t*Bu-C), 40.91 (2′-C), 62.04 (5′-C), 62.27 (5-CH_2O–C), 65.56, 67.58 (SEMCH_2O–C), 70.03 (NCH_2O–C), 71.68 (3′-C), 87.80 (1′-C), 87.88 (4′-C), 93.96 (OCH_2O–C), 110.24 (5-C), 138.05 (6-C), 150.86 (2-C), 162.03 (4-C). ESI-HRMS for $[C_{28}H_{56}N_2O_8Si_3+H]^+$ Calcd, 633.3423. Found, 633.3451.

3.0.21. 1-((2*R*,4*S*,5*R*)-4-((*tert*-Butyldimethylsilyl)oxy)-5-((*E*)-2-(methylsulfonyl)vinyl)tetrahydrofuran-2-yl)-5-(((2-(trimethylsilyl)ethoxy)methoxy)methyl)-3-((2-(trimethylsilyl)ethoxy)methyl)pyrimidine-2,4(1*H*,3*H*)-dione (37)

Following the procedure described for the synthesis of **14**, compound **36** (113 mg, 0.18 mmol) rendered **37** as white solid (33 mg, 29%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.00 (s, 9H, SEM $SiMe_3$), 0.02 (s, 9H, SEM $SiMe_3$), 0.10 (s, 3H, TBSSiMe), 0.10 (s, 3H, TBSSiMe), 0.90 (s, 9H, *t*Bu), 0.92–1.02 (s, 4H, SEMSiCH_2), 2.15–2.40 (m, 2H, 2′-C), 2.97 (s, 3H, SO_2Me), 3.58–3.75 (m, 4H, SEMCH_2O), 4.33 (dt, *J* = 6.4, 4.7 Hz, 1H, 4′-H), 4.40 (s, 2H, 5-CH_2O), 4.47 (td, *J* = 4.7, 1.8 Hz, 1H, 3′-H), 4.73 (s, 2H, OCH_2O), 5.39 (s, 2H, NCH_2O), 6.33 (t, *J* = 6.6 Hz, 1H, 1′-H), 6.69 (dd, *J* = 15.3 Hz, 1.8 Hz, 1H, 6′-H), 7.00 (1H, dd, *J* = 15 Hz, 4.2 Hz, 5′-H), 7.34 (s, 1H, 6-H). ESI-HRMS for $[C_{30}H_{58}N_2O_9SSi_3+Na]^+$ Calcd, 729.3069. Found, 729.1077.

3.0.22. 1-((2*R*,4*S*,5*R*)-4-((*tert*-Butyldimethylsilyl)oxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-2-yl)-3-(4-methoxybenzyl)-5-(((4-methoxybenzyl)oxy)methyl)-pyrimidine-2,4(1*H*,3*H*)-dione (38)

To a stirring solution of **33** (200 mg, 0.41 mmol) in anhydrous DMF (2 mL) at 0 °C under argon atmosphere was added 4-methoxybenzyl chloride (PMBCl, 167 μL, 1.23 mmol) followed by NaH (60% in mineral oil, 50 mg, 1.23 mmol) portion wise and stirred at room temperature for 4 h. Ethyl acetate (20 mL) and saturated aq.NH_4Cl (10 mL) was added. The aqueous layer was extracted with ethyl acetate (3 × 20 mL) and the combined organic layers were dried over anhyd Na_2SO_4, filtered, evaporated. The residue was purified by flash column chromatography (10% EtOAc in hexanes) to afford the title compound **38** as a white foam (40 mg, 13%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.05 (s, 3H, SiMe), 0.06 (s, 6H, SiMe), 0.07 (s, 3H, SiMe), 0.88 (s, 9H, *t*Bu), 0.89 (s, 9H, *t*Bu), 1.97 (ddd, *J* = 13.40, 7.69, 6.15 Hz, 1H, 2′-H), 2.26 (ddd, *J* = 13.18, 5.71, 2.49 Hz, 1H, 2′-H), 3.72–3.76 (m, 1H, 5′-H), 3.77 (s, 3H, PMB-OMe), 3.79 (s, 2H, PMB-OMe), 3.92 (q, *J* = 3.22 Hz, 1H, 5′-H), 4.18–4.33 (m, 2H, 5-CH_2O,) 4.37 (dt, *J* = 5.64, 2.60 Hz, 1H, 3′-H), 4.52 (s, 2H, PMB OCH_2), 4.95–5.13 (m, 2H, PMB NCH_2), 6.32 (dd, *J* = 7.91, 5.56 Hz, 1H, 1′-H), 6.82 (d, *J* = 8.79 Hz, 2H, Ar), 6.86 (d, *J* = 8.49 Hz, 2H, Ar), 7.26 (d, *J* = 8.49 Hz, 2H, Ar), 7.44 (d, *J* = 8.79 Hz, 2H, Ar), 7.59 (s, 1H, 6-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm −5.51, −5.43, −4.84, −4.66 (SiMe-C), 17.98, 18.39 (*t*Bu-tC), 25.74, 25.94 (*t*Bu-C), 41.17 (2′-C), 43.88 (NCH_2–C), 55.23, 55.25 (OMe–C), 63.01 (5′-C), 65.06 (5-CH_2O–C), 72.30 (3′-C), 72.81 (PMBCH_2O–C), 85.83 (1′-C), 87.80 (4′-C), 111.19 (5-C), 113.69, 113.80, 129.05, 129.51, 130.06, 130.73 (Ar-C) 135.99 (6-C), 150.80 (2-C), 159.04, 159.27 (Ar-C), 162.23 (2-C). ESI-HRMS for $[C_{38}H_{58}N_2O_8Si_2+H]^+$ Calcd, 727.3810. Found, 727.3797.

3.0.23. (1-((2*R*,4*S*,5*R*)-4-((*tert*-Butyldimethylsilyl)oxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-2-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)methyl pivalate (39)

To a solution of **33** (2.0 g, 4.1 mmol) in anhydrous pyridine (20 mL) and DMAP (100 mg) under inert atmosphere was added pivaloyl chloride (0.76 mL, 6.16 mmol) and the reaction mixture was stirred at room temperature overnight (18 h). Pyridine was evaporated under reduced pressure and the residue was purified by flash column chromatography (10–25% EtOAc in hexanes) to afford **39** as a white solid (2.0 g, 74%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.09 (s, 3H, SiMe), 0.09 (s, 2H, SiMe), 0.10 (s, 6H, $SiMe_2$), 0.90 (d, 9H, Si-*t*Bu), 0.91 (d, 9H, Si-*t*Bu), 1.19 (s, 9H, Piv-*t*Bu), 2.01 (ddd, *J* = 13.40, 7.69, 5.86 Hz, 1H, 2′-H), 2.32 (ddd, *J* = 13.18, 5.86, 2.64 Hz, 1H, 2′-H), 3.73–3.85 (m, 2H, 5′-H), 3.96 (q, *J* = 3.22 Hz, 1H, 4′-H), 4.40 (dt, *J* = 5.49, 2.67 Hz, 1H, 3′-H), 4.80 (s, 2H, 5-CH_2), 6.28 (dd, *J* = 7.91, 5.86 Hz, 1H, 1′-H), 7.78 (s, 1H, 6-H), 8.28 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm −5.42 (SiMe-C), -5.38 (SiMe-C), −4.83 (SiMe-C), −4.65 (SiMe-C), 18.01 (Si-*t*Bu-tC), 18.42 (Si-*t*Bu-tC), 25.74 (Si-*t*Bu-C), 25.93 (Si-*t*Bu-C), 27.15 (Piv-*t*Bu-C), 38.79 (Piv-*t*Bu-tC), 41.39 (2′-C), 59.07 (5-CH_2-C), 63.11 (5′-C), 72.36 (3′-C), 85.51 (1′-C), 88.09 (4′-C), 109.62 (5-C), 140.25 (6-C), 149.76 (2-C), 161.86 (4-C), 178.23 (Piv-C= O–C). ESI-HRMS for $[C_{27}H_{50}N_2O_7Si_2+H]^+$ Calcd, 571.3235. Found, 471.3242.

3.0.24. (1-((2*R*,4*S*,5*R*)-4-((*tert*-Butyldimethylsilyl)oxy)-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)methyl pivalate (40)

Following the procedure described for the synthesis of **12**, compound **39** (2.0 g, 3.05 mmol) gave compound **40** as a white solid (822 mg, 59%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.09 (s, 6H, SiMe), 0.90 (s, 9H,TBS-*t*Bu), 1.18 (s, 9H, Piv-*t*Bu), 2.19–2.38 (m, 2H, 2′-H), 2.94 (t, *J* = 5.32 Hz, 1H, 5′-OH), 3.70–3.81 (m, 1H, 5′-H), 3.90–3.99 (m, 2H, 5′ & 4′-H), 4.52 (dt, *J* = 6.25, 3.54 Hz, 1H, 3′-H), 4.83–4.94 (m, 2H, 5-CH_2O), 6.23 (t, *J* = 6.67 Hz, 1H, 1′-H), 8.08 (s, 1H, 6-H), 8.78 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm −4.86, −4.70 (SiMe-C), 17.98 (TBS *t*Bu-tC), 25.73 (*t*Bu-C), 27.04 (*t*Bu-C), 38.97 (piv *t*Bu-tC), 41.42 (2′-C), 58.63 (5-CH_2O-C), 62.06 (5′-C), 71.91 (3′-C), 86.60 (1′-C), 88.29 (4′-C), 109.26 (5-C), 142.71 (6-C), 149.70 (2-C), 161.95 (4-C), 179.70 (PivCO-C). ESI-HRMS for $[C_{21}H_{36}N_2O_7Si+H]^+$ Calcd, 457.2370. Found, 457.2414.

3.0.25. (1-((2*R*,4*S*,5*R*)-4-((*tert*-Butyldimethylsilyl)oxy)-5-(2-cyanovinyl)tetrahydrofuran-2-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)methyl pivalate (41)

Following the similar procedure to synthesize **13**, the reaction of compound **40** (0.8 g, 1.75 mmol), Dess–Martin reagent (0.48 M in CH_2Cl_2, 4.4 mL, 2.1 mmol) at room temperature for 7 h gave aldehyde as white foamy residue.

In a separate flask, to a solution of cyanomethyltriphenylphosphonium chloride (1.78 g, 5.25 mmol) in anhydrous THF (30 mL) at −78 °C under argon condition was added drop wise *n*-BuLi (1.6 M in hexanes, 3.3 mL, 5.25 mmol) and stirred for 30 min. To this ylide at −78 °C was added slowly a solution of above aldehyde in anhydrous THF (5 mL) and stirred at room temperature overnight. Following the similar workup procedure described for compound **19**, afforded **41** as a white solid (*E*-isomer–450 mg and *E* + *Z* mixture–240 mg, 82%). Data for *E*-isomer: ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.10 (s, 3H, SiMe), 0.11 (s, 3H, SiMe), 0.90 (s, 9H, TBS *t*Bu), 1.20 (s, 9H, Piv *t*Bu), 2.21–2.42 (m, 2H, 2′-H), 4.25–4.38 (m, 2H, 3′ & 4′-H), 4.81–4.95 (m, 2H, 5-CH_2O), 5.72 (dd, *J* = 16.40, 1.76 Hz, 1H, 6′-H), 6.24 (t, *J* = 6.44 Hz, 1H, 1′-H), 6.79 (dd, *J* = 16.26, 4.54 Hz, 1H, 5′-H), 7.54 (s, 1H, 6-H), 9.34 (br s, 1H, NH).

^{13}C NMR (75 MHz, $CDCl_3$) δ ppm −4.84, −4.66 (SiMe-C), 17.89 (TBS *t*Bu-tC), 25.63 (*t*Bu-C), 27.12 (*t*Bu-C), 38.91 (Piv *t*Bu-tC), 40.10 (2′-C), 58.36 (5-CH_2O-C), 74.53 (3′-C), 85.12 (4′-C), 86.07 (1′-C), 101.41 (6′-C), 110.38 (7′-C), 116.49 (5-C), 140.93 (6-C), 149.39 (5′-C), 149.70 (2-C), 162.32 (4-C), 178.84 (Piv CO–C). ESI-HRMS for $[C_{23}H_{35}N_3O_6Si+H]^+$ Calcd, 478.2373. Found, 478.2383.

3.0.26. (1-((2*R*,4*S*)-5-(2-cyanoethylidene)-4-hydroxytetrahydrofuran-2-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)methyl pivalate (42)

To a solution of compound **41** (65 mg, 0.136 mmol) in THF (1 mL) was added tetrabutylammonium hydroxide ($Bu_4N^+OH^-$, 40% in H_2O, 186 mg, 0.286 mmol) and stirred at room temperature for 5 h. Volatiles were evaporated under reduced pressure and the residue was purified by flash column chromatography (40–70% EtOAc in hexanes) to afford **42** as a white powder (20 mg, 40%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.19 (s, 9H, *t*Bu), 2.20 (d, *J* = 3.51 Hz, 1H, 3′-OH), 2.31 (dt, *J* = 13.84, 6.70 Hz, 1H, 2′-H), 2.56 (ddd, *J* = 14.06, 6.44, 2.64 Hz, 1H, 2′-H), 3.17 (dd, *J* = 7.03, 1.17 Hz, 2H, 6′-H), 4.77 (t, *J* = 7.03 Hz, 1H, 5′-H), 4.83–4.92 (m, 3H, 3′-H & 5-CH_2O-H), 6.62 (t, *J* = 6.74 Hz, 1H, 1′-H), 7.38 (s, 1H, 6-H), 8.46 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 13.70 (6-C), 27.10 (*t*Bu-C) 38.90 (*t*Bu-tC), 39.22 (2′-C), 58.48 (5-CH_2O-C), 70.25 (3′-C), 87.13 (1′-C), 89.96 (5′-C), 111.06 (5-C), 117.77 (7′-C), 139.55 (6-C), 149.40 (2-C), 159.39 (4-C), 161.42 (4′-C), 178.68 (Piv CO-C). ESI-HRMS for $[C_{17}H_{21}N_3O_6+H]^+$ Calcd, 364.1509. Found, 364.1455.

3.0.27. (1-((2*R*,4*S*,5*R*)-4-((*tert*-Butyldimethylsilyl)oxy)-5-(2-cyanoethyl)tetrahydrofuran-2-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)methyl pivalate (43)

To a solution of **41** (240 mg, 0.5 mmol) in ethylacetate (10 mL) was added 5%Pt-C (250 mg) and stream of hydrogen gas was bubbled through the reaction mixture for 4 h. The catalyst was filtered off and the filtrate was concentrated. The residue was purified by flash column chromatography (20–40% EtOAc in hexanes) to afford **43** as a white solid (170 mg, 70%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.09 (s, 3H, SiMe), 0.10 (s, 3H, SiMe), 0.90 (s, 9H, TBS *t*Bu), 1.20 (s, 9H, Piv *t*Bu), 1.94 (dddd, *J* = 13.88, 9.63, 7.54, 6.15 Hz, 1H, 5′-H), 2.02–2.17 (m, 1H, 5′-H), 2.23 (ddd, *J* = 13.40, 7.25, 5.71 Hz, 1H, 2′-H), 2.34 (ddd, *J* = 13.77, 7.03, 5.86 Hz, 1H, 2′-H), 2.43–2.65 (m, 2H, 6′-H), 3.85 (ddd, *J* = 9.37, 5.42, 3.66 Hz, 1H, 4′-H), 4.16 (dt, *J* = 11.13, 5.57 Hz, 1H, 3′-H), 4.88 (s, 2H, 5-CH_2O), 6.12 (dd, *J* = 6.74, 5.86 Hz, 1H, 1′-H), 7.55 (s, 1H, 6′-H), 9.13 (br s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm −4.88, −4.61 (SiMe-C), 14.22 (6′-C), 17.86 (TBS *t*Bu-tC), 25.64 (*t*Bu-C), 27.09 (*t*Bu-C), 28.96 (5′-C), 38.86 (Piv *t*Bu-tC), 40.48 (2′-C), 58.38 (5-CH_2O–C), 74.27 (3′-C), 84.26 (4′-C), 85.84 (1′-C), 109.99 (5-C), 118.95 (7′-C), 141.07 (6-C), 149.64 (2-C), 162.27 (4-C), 178.77 (Piv CO-C). ESI-HRMS for $[C_{23}H_{37}N_3O_6Si+H]^+$ Calcd, 480.2530. Found, 480.2535.

3.0.28. (1-((2*R*,4*S*,5*R*)-5-(2-cyanoethyl)-4-hydroxytetrahydrofuran-2-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)methyl pivalate (44)

Following the synthetic procedure described for compound **21**, compound **43** (80 mg, 0.17 mmol) afforded product **44** as a white solid (55 mg, 90%). ^{1}H NMR (300 MHz, CD_3OD) δ ppm 1.18 (s, 9H, *t*Bu), 1.92–2.06 (m, 1H, 5′-H) 2.06–2.16 (m, 1H, 5′-H), 2.29 (dd, *J* = 6.59, 5.71 Hz, 2H, 2′-H), 2.52–2.71 (m, 2H, 6′-H), 3.87 (dt, *J* = 9.01, 4.43 Hz, 1H, 4′-H), 4.17–4.24 (m, 1H, 3′-H), 4.84 (s, 2H, 5-CH_2O), 6.20 (t, *J* = 6.59 Hz, 1H, 1′-H), 7.78 (s,1H, 6-H). ^{13}C NMR (75 MHz, CD_3OD) δ ppm 14.51 (6′-C), 27.53 (*t*Bu-C), 30.37(5′-C), 39.90 (*t*Bu-tC), 40.30 (2′-C), 60.22 (5-CH_2O), 74.70 (3′-C), 86.05 (4′-C), 86.85 (1′-C), 110.70 (5-C), 120.82 (7′-C), 143.01 (6-C), 151.87 (2-C), 164.85 (4-C), 180.09 (Piv CO-C). ESI-HRMS for $[C_{17}H_{23}N_3O_6 + H]^+$ Calcd, 366.1665. Found, 366.1631.

3.0.29. 3-((2*R*,3*S*,5*R*)-3-hydroxy-5-(5-(hydroxymethyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-2-yl)propanenitrile (30)

A solution of 0.5 M NaOMe in methanol (0.7 mL, 0.342 mmol) was added to compound **44** (25 mg, 0.068 mmol) and stirred at room temperature for 3 h. Acetic acid (30 μL, 0.5 mmol) was added and the solvents were evaporated. The residue was purified by flash column chromatography (4–8% MeOH in CH_2Cl_2) to afford **30** as a white solid (19 mg, 95%). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.76–1.90 (m, 1H, 5′-H), 1.90–2.03 (m, 1H, 5′-H), 2.06–2.25 (m, 2H, 2′-H), 2.53–2.68 (m, 2H, 6′-H), 3.72 (dt, *J* = 8.49, 4.54 Hz, 1H, 4′-H), 4.05–4.13 (m, 1H, 3′-H), 4.16 (s, 2H, 5-CH_2O), 4.97 (br s, 1H, 5-MeOH), 5.39 (br s, 1H, 3′-OH), 6.16 (t, *J* = 6.74 Hz, 1H, 1′-H), 7.42 (s, 1H, 6-H), 11.34 (br s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 13.21 (6′-C), 28.53 (5′-C), 38.36 (2′-C), 55.70 (5-CH_2O–C), 72.53 (3′-C), 83.87 (1′-C), 83.97 (4′-C), 114.46 (5-C), 120.17 (7′-C), 136.38 (6-C), 150.13 (2-C), 162.42 (4-C). ESI-HRMS for $[C_{12}H_{15}N_3O_5+HCOO^-]^-$ Calcd, 326.0988. Found, 326.0985.

3.0.30. (1-((2*R*,4*S*,5*R*)-5-(2-(1*H*-tetrazol-5-yl)ethyl)-4-((*tert*-Butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)methyl pivalate (45)

Following the procedure described for the synthesis of **22**, compound **43** (80 mg, 0.17 mmol) rendered **45** as white solid (74 mg, 74%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.08 (s, 6H, SiMe), 0.89 (s, 9H, TBS *t*Bu), 1.24 (s, 9H, Piv *t*Bu), 1.74–1.91 (m, 1H, 5′-H), 2.04–2.17 (m, 1H, 2′-H), 2.19–2.33 (m, 1H, 5′-H), 2.39 (ddd, *J* = 13.55, 5.93, 3.95 Hz, 1H, 2′-H), 3.13–3.28 (m, 2H, 6′-H), 4.03 (dt, *J* = 10.69, 3.00 Hz, 1H, 4′-H), 4.10–4.18 (m, 1H, 3′-H), 4.93–5.08 (m, 2H, 5-CH_2O), 6.21 (t, *J* = 6.30 Hz, 1H, 1′-H), 7.79 (s, 1H, 6-H), 8.99 (br s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm −4.83, −4.66 (SiMe-C), 17.95 (TBS *t*Bu-tC), 20.40 (6′-C), 25.68 (*t*Bu-C), 27.12 (*t*Bu-C), 33.34 (5′-C), 39.38 (Piv *t*Bu-tC), 40.56 (2′-C), 59.14 (5-CH_2O–C), 75.13 (3′-C), 86.66 (4′-C), 87.00 (1′-C), 109.27 (5-C), 142.20 (6-C), 149.73 (2-C), 155.28 (7′-C), 162.27 (4-C), 182.48 (Piv CO-C). ESI-HRMS for $[C_{23}H_{38}N_6O_6Si-H]^-$ Calcd, 521.2544. Found, 521.2551.

3.0.31. 1-((2*R*,4*S*,5*R*)-5-(2-(1*H*-tetrazol-5-yl)ethyl)-4-((tert-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)-5-(hydroxymethyl)pyrimidine-2,4(1*H*,3*H*)-dione (46)

Following the procedure described for the synthesis of **30**, compound **45** (90 mg, 0.17 mmol) after chromatography (5–7% MeOH + 0.5% HCOOH in CH_2Cl_2) rendered **46** as white solid (70 mg, 92%). ^{1}H NMR (300 MHz, CD_3OD) δ ppm 0.11 (s, 6H, SiMe), 0.91 (s, 9H, *t*Bu), 2.00–2.12 (m, 1H, 5′-H), 2.12–2.38 (m, 3H, 2′ & 5′-H), 2.99–3.19 (m, 2H, 6′-H), 3.82 (dt, *J* = 9.37, 4.10 Hz, 1H, 4′-H), 4.31 (dt, *J* = 6.00, 4.32 Hz, 1H, 3′-H), 4.35 (d, *J* = 1.17 Hz, 2H, 5-CH_2O), 6.20 (t, *J* = 6.74 Hz, 1H, 1′-H), 7.53 (s, 1H, 6-H). ^{13}C NMR (75 MHz, CD_3OD) δ ppm −4.70, −4.50 (SiMe-C), 18.80 (*t*Bu-tC), 21.38 (6′-C), 26.25 (*t*Bu-C), 32.52 (5′-C), 40.87 (2′-C), 57.80 (5-CH_2O–C), 76.34 (3′-C), 86.61 (1′-C), 87.00 (4′-C), 115.70 (5-C), 138.70 (6-C), 152.12 (2-C), 158.26 (7′-C), 165.00 (4-C). ESI-HRMS for $[C_{18}H_{30}N_6O_5Si-H]^-$ Calcd, 437.1969. Found, 437.1982.

3.0.32. 1-((2*R*,4*S*,5*R*)-5-(2-(1*H*-tetrazol-5-yl)ethyl)-4-hydroxytetrahydrofuran-2-yl)-5-(hydroxymethyl)pyrimidine-2,4(1*H*,3*H*)-dione (47)

In a polypropylene vessel, to a solution of compound **46** (70 mg, 0.16 mmol) in methanol (10 mL) was added NH_4F (120 mg,

3.2 mmol) and stirred at 50 °C for 2 days. Dichloromethane (10 mL) was added and filtered. The filtrate was concentrated and the residue purified by flash column chromatography (8–12% MeOH + 0.5% HCOOH in CH_2Cl_2) to afford **47** as a white solid (45 mg, 87%).^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.88–2.01 (m, 1H, 5′-H), 2.01–2.24 (m, 3H, 5′ & 2′-H), 2.86–3.05 (m, 2H, 6′-H), 3.65–3.75 (m, 1H, 4′-H), 4.05–4.13 (m, 1H, 3′-H), 4.16 (d, J = 0.88 Hz, 2H, 5-CH_2O), 6.15 (t, J = 6.74 Hz, 1H, 1′-H), 7.44 (s, 1H, 6-H), 11.35 (br s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 19.65 (6′-C), 30.89 (5′-C), 38.63 (2′-C), 55.69 (5-CH_2–C), 72.77 (3′-C), 83.60 (1′-C), 84.50 (4′-C), 114.48 (5-C), 136.18 (6-C), 150.14 (2-C), 155.96 (7′-C), 162.42 (4-C). ESI-HRMS for $[C_{12}H_{16}N_6O_5-H]^-$ Calcd, 323.1104. Found, 323.1234.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.10.018.

References and notes

1. (a) Mdluli, K.; Spigelman, M. *Curr. Opin. Pharmacol.* **2006**, *6*, 459; (b) Lamichhane, G. *Trends Mol. Med.* **2011**, *17*, 25.
2. (a) Villemagne, B.; Crauste, C.; Flipo, M.; Baulard, A. R.; Déprez, B.; Willand, N. *Eur. J. Med. Chem.* **2012**, *51*, 1; (b) Koul, A.; Arnoult, E.; Lounis, N.; Guillemont, J.; Andries, K. *Nature* **2011**, *469*, 483.
3. Keating, T. A.; Newman, J. V.; Olivier, N. B.; Otterson, L. G.; Andrews, B.; Boriack-Sjodin, P. A.; Breen, J. N.; Doig, P.; Dumas, J.; Gangl, E.; Green, O. M.; Guler, S. Y.; Hentemann, M. F.; Joseph-McCarthy, D.; Kawatkar, S.; Kutschke, A.; Loch, J. T.; McKenzie, A. R.; Pradeepan, S.; Prasad, S.; Martínez-Botella, G. *ACS Chem. Biol.* **2012**.
4. (a) Van Calenbergh, S.; Pochet, S.; Munier-Lehmann, H. *Curr. Top. Med. Chem.* **2012**, *12*, 694; (b) Van Poecke, S.; Munier-Lehmann, H.; Helynck, O.; Froeyen, M.; Van Calenbergh, S. *Bioorg. Med. Chem.* **2011**, *19*, 7603.
5. Haouz, A.; Vanheusden, V.; Munier-Lehmann, H.; Froeyen, M.; Herdewijn, P.; Van Calenbergh, S.; Delarue, M. *J. Biol. Chem.* **2003**, *278*, 4963.
6. Frecer, V.; Seneci, P.; Miertus, S. *J. Comput. Aided Mol. Des.* **2011**, *25*, 31.
7. Amewu, R.; O'Neill, P. M.; Stachulski, A.; Ellis, G.; Ward, S. A. WO 2008/038030.
8. Mazur, W. A.; He, Y.; Sorekin, V. WO 2010/072831.
9. Song, Y.; Liu, C.-I.; Lin, F. Y.; No, J. H.; Hensler, M.; Liu, Y. L.; Jeng, W.; Low, J.; Liu, G. Y.; Nizet, V.; Wang, A. J-H.; Oldfield, E. *J. Med. Chem.* **2009**, *52*, 3869.
10. Grover, R. K.; Pond, S. J. K.; Cui, Q.; Subramaniam, P.; Case, D. A.; Millar, D. P.; Wentworth, P., Jr. *Angew. Chem., Int. Ed.* **2007**, *46*, 2839.
11. Evano, G.; Schaus, J. V.; Panek, J. S. *Org. Lett.* **2004**, *6*, 525.
12. (a) Satoh, T.; Nanba, K.; Suzuki, S. *Chem. Pharm. Bull.* **1971**, *19*, 817; (b) Yadav, J. S.; Yadav, N. N.; Rao, T. S.; Reddy, B. V. S.; Ghamdi, A. A. K. A. *Eur. J. Org. Chem.* **2011**, 4603.
13. Rhodes, R. A.; Boykin, D. W. *Synth. Commun.* **1988**, *18*, 681.
14. Saneyoshi, H.; Tamaki, K.; Ohkubo, A.; Seio, K.; Sekine, M. *Tetrahedron* **2008**, *64*, 4370.
15. Kaburagi, Y.; Kishi, Y. *Org. Lett.* **2007**, *9*, 723.
16. (a) El Safadi, Y.; Paillart, J.-C.; Laumond, G.; Aubertin, A.-M.; Burger, A.; Marquet, R.; Vivet-Boudou, V. *J. Med. Chem.* **2010**, *53*, 1534; (b) Shiau, G. T.; Schinazi, R. F.; Chen, M. S.; Prusoff, W. H. *J. Med. Chem.* **1980**, *23*, 127; (c) Conte, M. R.; Galeone, A.; Avizonis, D.; Hsu, V. L.; Mayol, L.; Kearns, D. R. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 79.
17. Rodrigues-Correia, A.; Koeppel, M. B.; Schäfer, F.; Joshi, K. B.; Mack, T.; Heckel, A. *Anal. Bioanal. Chem.* **2011**, *399*, 441.
18. de Kort, M.; de Visser, P. C.; Kurzeck, J.; Meeuwenoord, N. J.; van der Marel, G. A.; Rüger, W.; van Boom, J. H. *Eur. J. Org. Chem.* **2001**, 2075.
19. Tronchet, J. M. J.; Chalard, F.; Rivara-Minten, E.; Seman, M.; De Clercq, E.; Balzarini, J.; Dilda, P. *Nucleosides Nucleotides Nucleic Acids* **2002**, *21*, 191.