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Novel bicyclic sugar modified nucleosides: synthesis, conformational analysis and antiviral evaluation

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Abstract—Methodology previously described by us was applied to the formation of novel conformationally restrained bicyclic sugar modified nucleosides, with introduction of an oxazole and a thiocarbamate ring at the 2′,3′-positions of the ribonucleosides. Two novel alkyl derivatives of 2′,3′-dideoxy-2′,3′-oxazole-β-D-uridine and a novel uridine 2′,3′-thiocarbamate were successfully synthesised. Conformational evaluation of all the synthesised compounds was conducted using the theoretical potential energy calculation via the MACROMODEL V.6.0 molecular modelling programme. The conformationally restrained nucleosides described were evaluated against a wide range of DNA and RNA viruses. None of the compounds showed specific antiviral effects at subtoxic concentrations.
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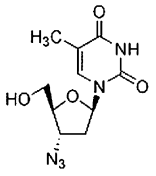
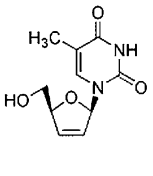
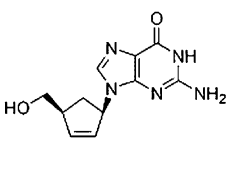
1. Introduction

In recent years, many nucleosides with modifications in the sugar unit have been described with the hope of obtaining new antiviral and anticancer agents.[1–3] In the treatment of AIDS, the use of 2′,3′-dideoxynucleosides such as zidovudine (**1**, AZT)[4] and stavudine (**2**, d4T),[5] have been approved and used clinically as monotherapy as well as in combination therapy with other antivirals [highly active antiretroviral therapy (HAART)]. However, their long-term usefulness is limited owing to the development of resistant strains and toxicity issues;[6] thus, there is a continuous need for improved antiviral drugs and for a better understanding of the structure–activity relationship (SAR) for different nucleoside mimetics and their pharmacological effects.


(**1**) Zidovudine, AZT


(**2**) Stavudine, d4T


(**3**) Carbovir

Keywords: Conformationally restrained nucleosides; Oxazole; Thiocarbamate; Conformational analysis; Antiviral activity.
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The conformational behaviour of natural as well as modified nucleosides is of immense importance for their biological activity. Soon after the first successful clinical use of AZT (**1**), the conformational features of the RT inhibitors were correlated to their biological properties with the intent of using this information as a paradigm for the development of other related antiretroviral nucleosides.[7] Any SAR study of an antiretroviral nucleoside is complicated by the complexity of the anabolic process of activation, which involves three sequential steps to convert the nucleoside to its 5′-triphosphate (NTP), and the final interaction of the NTP with the target enzyme, RT. Therefore, conformational preferences exhibited by the nucleoside or its nucleotides, must be identified at each intervening enzymatic step. The only variant step, common to all nucleoside RT inhibitors, is the final interaction of the NTP with RT. The route to the NTP anabolite, on the other hand, involves different cellular kinases all of which are highly dependent on the nature of the heterocyclic base.[8]

Van Roey et al.[10] focused on the activation process and assumed that a similar conformation of the sugar moiety was required for all the steps leading to the NTP anabolite. From their conformational analysis, it was therefore generalised that nucleoside analogues with a favourable C3′-*exo* (*South*-type, $_3$E) conformation, which is strongly associated with an *ap* torsion angle γ about the C4′–C5′ bond, were proposed to be the best

substrates to generate abundant pools of the NTP anabolite. NMR studies of the triphosphate of AZT (AZTTP) and the target viral enzyme HIV-1 RT showed that AZTTP binds to the enzyme with a pseudorotational angle, $P \approx 60°$ and the natural substrate thymidine triphosphate (TTP) binds with $P \approx 55°$; both of these are C4′-*exo* conformations, which, in a broad definition, can be recognised as *North*-type conformations (Fig. 1).[11]

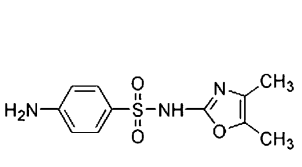
Due to the low energy barrier (≈2 kcal/mol) between the two dominating conformers, C3′-*endo North*-type, ^{3}E) and C2′-*endo* (*South*-type, ^{2}E), a fast equilibrium between the *North* or *South*-type states exists; thus the conformation of the nucleoside in solution can differ sharply from that determined in the solid state. This is because in solid state, only one of the two solution conformations is present, and its selection is additionally determined by crystal packing forces.[12] Consequently, any conformation–activity study based exclusively on solid-state conformational parameters would be flawed unless both solution and solid-state conformations are known to be equivalent.

It is for the reasons above that conformationally restrained (restricted or locked) nucleosides (sometimes termed as locked nucleic acids—LNA) have drawn considerable attention since these nucleosides adopt certain restricted, geometrical shapes, which can be useful biological tools in the evaluation of conformational preferences of nucleos(t)ide-converting enzymes and in studies on the interactions of the nucleos(t)ide with corresponding receptors and enzymes.[13,14]

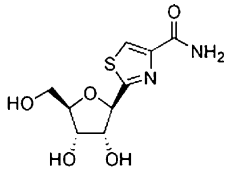
Furthermore, in recent years many more conformationally restrained nucleosides have been synthesised as potential antiviral agents. Partially rigid 2′,3′-didehydro-2′,3′-dideoxyribonucleoside analogues such as carbovir (**3**)[15] and d4T (**2**), which have shown potent antiviral activities against HIV, may be considered the earlier examples in this class. The molecular conformation of d4T showed that the 2′,3′-double bond eliminates ring puckering, resulting in a nearly planar conformation corresponding to the O4′-*endo* envelope conformation with a high-*anti* glycosidic bond conformation.[16]

A number of studies involving conformationally restricted bicyclic nucleoside analogues have been described[17–20] and these results have led to the conclusion that the active anti-HIV nucleoside analogues must display an appropriate flexibility in order to adopt a *South*-type conformation for phosphorylation and a *North*-type conformation for inhibiting HIV-1 RT.

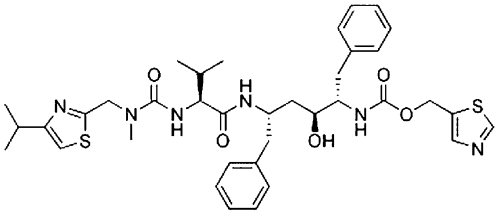
Thiazole and oxazole heterocycles of biological and medicinal interest are found naturally, which display antibiotic, antitumour, antifungal and antiviral activities.[21] The interesting biological activity combined with the beneficial pharmacokinetic profiles of oxazoles and thiazoles has resulted in their introduction into a wide range of therapeutic agents, including antibacterial (e.g., the sulfonamide, sulfamoxol **4**),[22] anticancer (e.g., the *C*-nucleoside, tiazofurin **5**)[23] and antiviral (e.g., the protease inhibitor, ritonavir **6**) agents.[24] It was therefore of interest to incorporate these heterocycles into conformationally restrained bicyclic nucleosides.



(**4**) Sulphamoxol



(**5**) Tiazofurin



(**6**) Ritonavir

This paper will describe the formation and synthesis of the restrained bicyclic nucleosides substituted at the 2′,3′-positions of the ribose ring with oxazole and thiazole heterocycles, which could mimic the d4T rigid structure, with the C1′–C2′–C3′–C4′ moiety adopting a locked conformation (Fig. 2). 2′-Amino-2′-deoxynucleosides are of interest as both antiviral agents[25,26] and as tools for probing biochemical mechanisms such as ribozyme catalysis,[27] therefore the fusion of the bicyclic ring structure was designed to accommodate a 2′-amino-2′-deoxy substitution to determine the effect of such

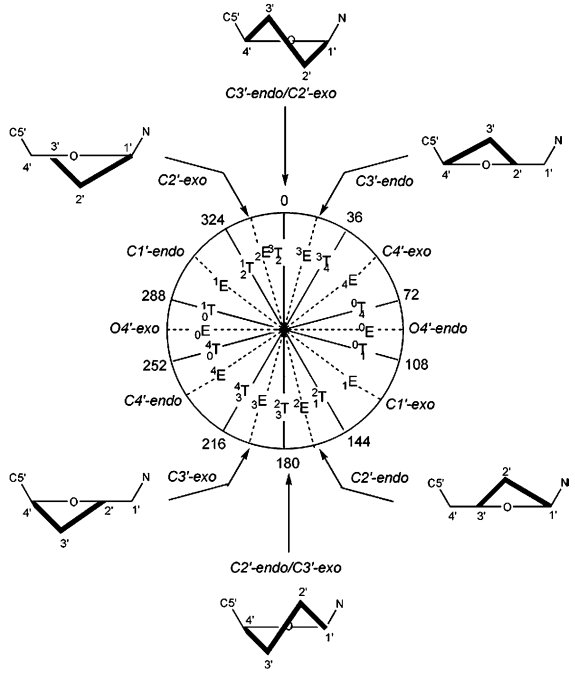


Figure 1. Pseudorotational cycle of the furanose ring in nucleosides (E = envelope, T = twist).[9]

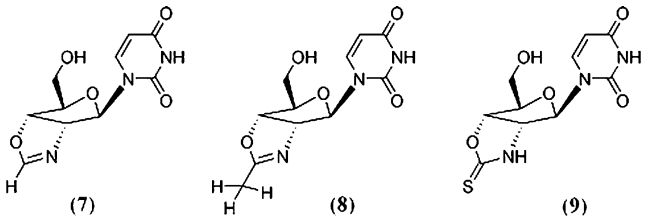


Figure 2. Structures of the target compounds.

a substitution on both conformation and antiviral activity.

A degree of conformational flexibility has been hypothesised to be required and it was hoped that the target compounds would meet the 'generalised' requirement.

These conformationally restricted nucleosides and/or their triphosphates must be recognised by the cellular phosphorylating enzymes and by HIV-1 RT (or by the phosphorylating kinases of DNA viruses such as herpes simplex virus and varicella-zoster virus. From literatures searches, to date no such compounds have been synthesised before by other research groups.

2. Chemistry

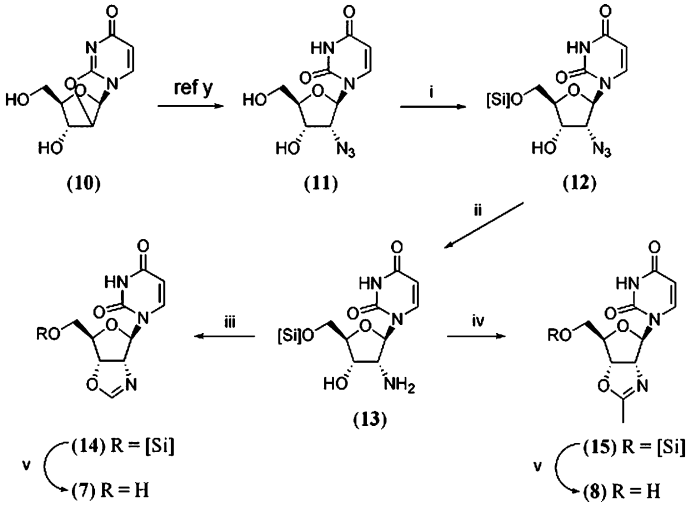
Methodology previously established by our group was applied to the synthesis of these target 2′,3′-oxazole fused bicyclic nucleosides.[28] 2′-Azido-2′-deoxyuridine (**11**) was readily prepared from 2,2′-anhydrouridine (**10**) using the method described by Kirschenheuter et al.,[29] which employed in situ generation of lithium azide from lithium fluoride and azidotrimethylsilane in the presence of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) in DMF as co-solvent (1:1 v/v) at 100–110 °C (Scheme 1).

The 2′-azido-2′-deoxyuridine (**11**) was selectively protected using *t*-BDMSCl in the presence of imidazole catalyst in DMF using the established procedure for 2 h at room temperature (Scheme 1)[30] with good yields obtained after purification by flash column chromatography. The 5′-protected product (**12**) was subsequently subjected to the Staudinger reaction,[31] involving reaction with triphenyl phosphine in THF at room temperature, followed by the addition of water, resulting in reduction of the azido moiety to the required 2′-amino-2′-deoxy derivative (**13**) (Scheme 1).

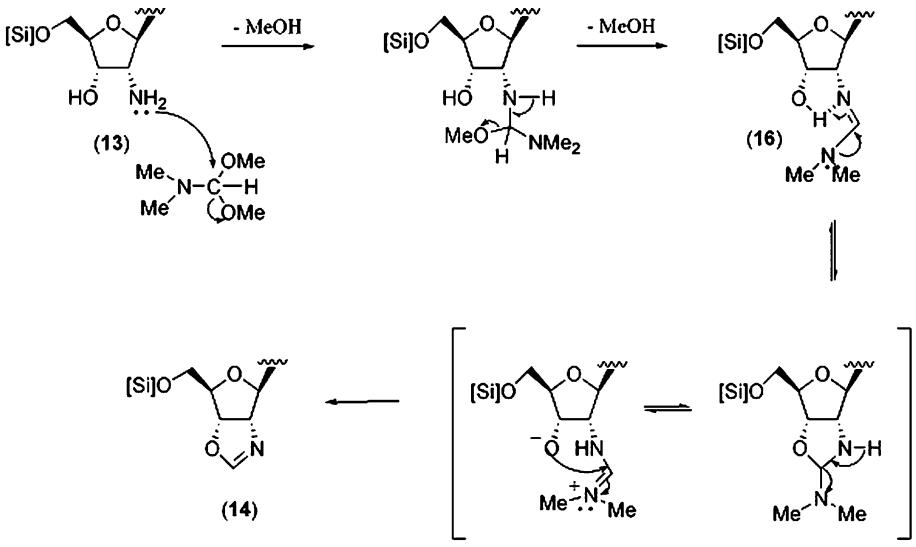
The next step was to cyclise the amine derivative (**13**) to the 2′,3′-oxazole nucleoside (**14**) using DMFDMA in DMF at room temperature overnight. During monitoring of the reaction a new product, which was more polar than the final oxazole product, was observed after a few hours by TLC. Mass spectroscopy of the crude reaction mixture after 3 h indicated a molecular ion of 413.3 $(M+H)^+$, which corresponded with the intermediate structure (**16**) shown in Scheme 2, confirming the previously reported mechanism of the oxazole cyclisation reaction.[28]

Owing to the instability of the bicyclic 2′,3′-oxazole product (**14**) towards acidic conditions (silica used during purification), it was essential to add 1% triethylamine (Et_3N) to the eluent to neutralise the solvent system during purification. The product (**14**) was isolated in 78% yield with formation of the desired compound characterised by the appearance of the imine proton signal in the ^1H NMR spectrum downfield at 7.00 ppm as a singlet and the imine carbon signal at 156.08 ppm in the ^{13}C NMR spectrum.

Deprotection of the silyl protecting group with TBAF in THF at room temperature gave the target compound 2′,3′-dideoxy-2′,3′-oxazole-β-D-uridine (**7**) in 83% yield. In a similar manner 5′-*O*-TBDMS-2′-amino-2′-deoxyuridine (**13**) was treated with *N*,*N*-dimethylacetamide dimethylacetal (DMADMA) to form the 5′-*O*-TBDMS-2′,3′-oxazole nucleoside (**15**), which was deprotected with TBAF to give 2′,3′-dideoxy-2′,3′-(2-methyloxazole)-β-D-uridine (**8**).



Scheme 1. Reagents and conditions: (i) $^tBuMe_2SiCl$, imidazole, DMF, 2 h (70%) (ii) Ph_3P, THF, H_2O, 60 °C, 2 h (87%) (iii) DMFDMA, DMF, o/n (78%) (iv) DMADMA, DMF, o/n (60%) (v) TBAF, THF, 1 h (**7**, 83%) (**8**, 86%).



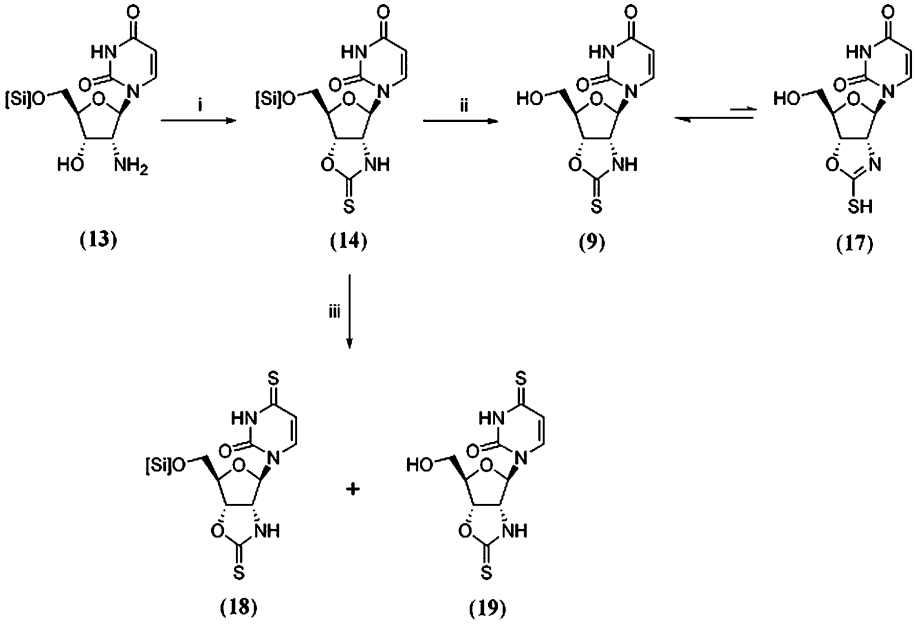
Scheme 2. The synthesis of the novel bicyclic oxazole nucleoside by the established method of Molina and Simons[28] and its proposed mechanism.

Reaction of **13** with thiocarbonyldiimidazole in dichloromethane at room temperature produced the thiocarbamate (**17**) in high yield (94%) as a white solid with melting point 230–234 °C. The compound was characterised by the appearance of the NH proton of the thiocarbamate at ~10 ppm in the ^{1}H NMR spectrum and the thiocarbonyl C=S was noted at 189.19 ppm in the ^{13}C NMR. Deprotection of (**17**) using TBAF in THF afforded the major product as the stable isomer (**9**), and the minor tautomeric product (**17**), as indicated by ^{1}H NMR and ^{13}C NMR spectroscopy. Low resolution mass spectrometry ES (+ve) indicated a molecular ion at 308.0 $(M+Na)^{+}$ and EI (+ve) indicated a molecular ion at 285.1 $(M+H)^{+}$ (Scheme 3).

Treatment of (**14**) with Lawesson's reagent[32] at 80 °C for 4 h followed by purification by column chromatography gave the silyl protected nucleoside (**18**) in 34% yield and the deprotected product 2′,3′-dideoxy-2′,3′-[3,4-*d*](oxazolidin-2-thione)-β-D-4-thiouridine (**19**) in 66% yield. ^{13}C NMR confirmed the presence of two thiocarbonyl groups resonating at δ 187.8 and 190.9.

3. Conformational analysis

In this study, an *internal coordinate Monte Carlo*[33] was chosen as an appropriate method, owing to the ability to search global conformation on symmetrical and unsymmetrical molecules having up to a dozen variable torsion angles. Also, the nucleoside has internal freedom of rotation about inter-atomic bonds, such rotations occur about the glycosyl bond (χ), and internal (ν_0–ν_4) and external ($C_{4'}$–$C_{5'}$) (γ) sugar ring bonds. The structure minimisation was done as a single operation by the



Scheme 3. Reagents and conditions: (i) $(Im)_2C$=S, CH_2Cl_2, 5 h (94%) (ii) TBAF, THF, 8 h (72%) (iii) Lawesson's reagent, toluene, 80 °C, 4 h (18, 34%; 19, 66%).

BATCHMIN software, a noninteractive molecular mechanics programme, used as part of the MACROMODEL V.60 interactive molecular modelling programme. BATCHMIN was used for all energy calculations including energy minimisation and conformational searching. MACROMODEL V.60 program was run on a Silicon Graphics workstation.

Generally, for all the structures shown, the conformational search was done with 1000 MC steps, with water as the solvent. For the conformationally restrained nucleoside (**7**) shown in Figure 3, the energy minimisation was performed using water as the chosen solvent.

Conformational search for **7** gave 29 unique conformations out of the 1000 conformers processed, with one stable conformer with a global minimum energy, $E = -346.93$ KJ/mol found 102 times in the search. The analysis data for this stable conformer is shown in Table 1.

Similarly data for the stable conformers of the bicyclic nucleosides (**8**) (Fig. 4) and (**9**) (Fig. 5) are shown in Tables 2 and 3.

Using the equations described by Altona and Sundaralingam[34] (Eqs. 1 and 2) and the torsion angles obtained (Tables 1–3) the pseudorotation phase angle, P and the maximum torsion angle (degree of pucker), v_{max} can be determined (Table 4). In nucleosides, where the furanose ring is unsymmetrically substituted, potential energy thresholds are created, which limit the pseudorotation and lead to preferred puckering modes.

$$\mathrm{Tan}P = \frac{(v_4 + v_1) - (v_3 + v_0)}{2[v_2(\sin 36^\circ + \sin 72^\circ)]} \quad (1)$$

$$v_{max} = \frac{v_2}{\mathrm{Cos}P} \quad (2)$$

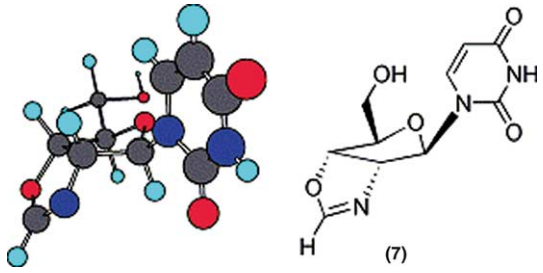


Figure 3. The 3D structure of the novel bicyclic nucleoside **7** and its corresponding chemical structure.

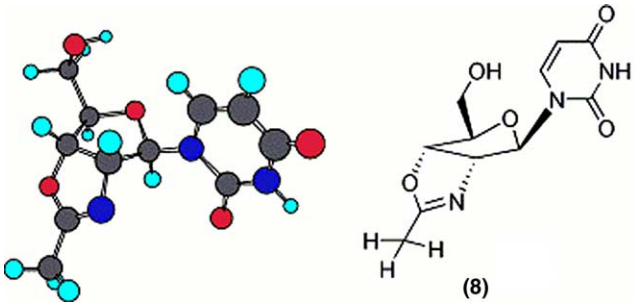


Figure 4. The 3D structure of compound **8** and its corresponding chemical structure.

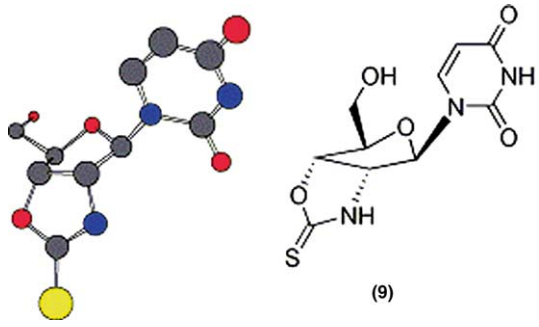


Figure 5. The 3D structure of structure of uridine 2′,3′-thiocarbamates (**9**) and its corresponding structure.

For compound (**7**) $P = 89°$, that is, it has a stable conformation of $O_{4'}$-*endo* (*East* conformation), the intermediate between *North* and *South* conformations. This conformation although unusual as compared with the conformers of the naturally occurring nucleosides (*North* and *South* conformation), has similarity to the conformationally bicyclic nucleosides synthesised by Nielsen et al.[35] Although the P value of compound (**8**) varied by nearly 20° from compound (**7**), the conformation can be considered near the *East* conformation. However, the stable conformer can now be described as a near twist conformation, that is, $O_{4'}$-*endo*-$C_{1'}$-*exo* twist, which is designated as $^0{}_1$T in the pseudorotation cycle. The thiocarbamate (**9**), with $P = 72.55$, also displayed an *East* conformation, described as $O_{4'}$-*endo*-$C_{4'}$-*exo* twist conformations, which can also be designated as $^0{}_4$T in the cycle (Table 4).

For the orientation about the glycosyl link $C_{1'}$–$N_{1'}$, the *syn–anti* conformations can be derived from the torsion angle about that bond, χ. From Table 1, $\chi = -135.4°$, it can be deduced that (**7**) has an *anti* conformation. The

Table 1. The internal coordinates of the stable conformer **7** with $E = -346.93$ KJ/mol

Atomic distance (Å)	Bond angle (°)	Torsion angle (°)	Torsion angle (°)
C1′–C2′ = 1.535	O1′–C1′–C2′ = 103.2	$\upsilon_0 = -41.0$	$\gamma = 179.9$
C2′–C3′ = 1.530	C1′–C2′–C3′ = 104.6	$\upsilon_1 = 23.5$	$\delta = 104.0$
C3′–C4′ = 1.523	C2′–C3′–C4′ = 103.6	$\upsilon_2 = 0.5$	$\chi = -135.4$
C4′–O1′ = 1.417	C3′–C4′–O1′ = 104.3	$\upsilon_3 = -24.5$	
O1′–C1′ = 1.422	C4′–O1′–C1′ = 106.7	$\upsilon_4 = 41.8$	
C1′–N1 = 1.455			

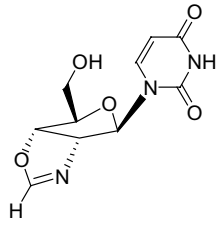
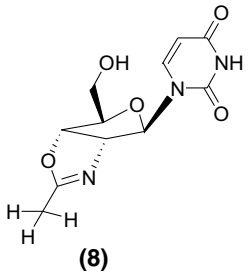
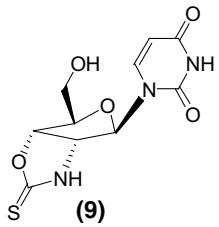
Table 2. The internal coordinates of the stable conformer **8** with $E = -349.20$ KJ/mol

Atomic distance (Å)	Bond angle (°)	Torsion angle (°)	Torsion angle (°)
C1′–C2′ = 1.532	O1′–C1′–C2′ = 102.6	$\upsilon_0 = -42.0$	$\gamma = 60.8$
C2′–C3′ = 1.527	C1′–C2′–C3′ = 103.3	$\upsilon_1 = 33.6$	$\delta = 120.9$
C3′–C4′ = 1.524	C2′–C3′–C4′ = 104.3	$\upsilon_2 = -14.2$	$\chi = -134.2$
C4′–O1′ = 1.417	C3′–C4′–O1′ = 105.8	$\upsilon_3 = -9.9$	
O1′–C1′ = 1.417	C4′–O1′–C1′ = 107.6	$\upsilon_4 = 33.1$	
C1′–N1 = 1.455			

Table 3. The internal coordinates of the stable conformer (**9**) with $E = -144.47$ KJ/mol

Atomic distance (Å)	Bond angle (°)	Torsion angle (°)	Torsion angle (°)
C1′–C2′ = 1.539	O1′–C1′–C2′ = 104.2	$\upsilon_0 = -35.1$	$\gamma = 179.7$
C2′–C3′ = 1.537	C1′–C2′–C3′ = 105.2	$\upsilon_1 = 12.1$	$\delta = 92.4$
C3′–C4′ = 1.522	C2′–C3′–C4′ = 102.0	$\upsilon_2 = 12.8$	$\chi = -134.7$
C4′–O1′ = 1.416	C3′–C4′–O1′ = 103.8	$\upsilon_3 = -33.8$	
O1′–C1′ = 1.423	C4′–O1′–C1′ = 114.8	$\upsilon_4 = 44.3$	
C1′–N1 = 1.456			

Table 4. Summarised conformations of the global minimum of the novel bicyclic nucleosides (**7**), (**8**) and (**9**)

	Bicyclic Nucleoside		
Conformations	 **(7)**	 **(8)**	 **(9)**
P Value	89°	110°	73°
Sugar Pucker	$O_{4'}$-*endo* (*East*)	$O_{4'}$-*endo*-$C_{1'}$-*exo* twist (*East*)	$O_{4'}$-*endo*-$C_{4'}$-*exo* twist (*East*)
syn/*anti*	*anti*	*anti*	*anti*
$C_{4'}$–$C_{5'}$ bond	*ap* (*trans*) = 180°	+*sc* (*gauche*) = 60	*ap* (*trans*) = 180°

anti conformation is the preferred conformation for pyrimidine nucleosides as there is no particular steric hindrance between the sugar and the base. Compounds (**8**) and (**9**) also displayed an *anti* conformations about the glycosyl link $C_{1'}$–$N_{1'}$, with values of $\chi = -134.2°$ and $-134.7°$, respectively (Tables 2 and 3).

Orientation about the $C_{4'}$–$C_{5'}$ bond can also be derived from the torsion angle about that bond, γ. Compound (**7**) can be described as having an *ap* (*trans*) conformation, this assumes that the rotation about the exocyclic $C_{4'}$–$C_{5'}$ bond is 179.9° (≈180°) relative to the sugar ring (Table 1). Although pyrimidine nucleosides would normally prefer the +*sc* ($\gamma = 60°$) conformations, in this case the sugar puckering of the nucleoside (**7**), having an $O_{4'}$-*endo* envelope conformation, may well be explained by the $O_{4'}$ being 'above' the planar of the other four atoms in the sugar ring; thus, this may cause repulsive interactions and direct the $O_{5'}$ 'away' from the ribose ring by approximately 180° (*trans*). The *ap* (*trans*) conformation was also observed for the thiocarbamate (**9**), $\gamma = 179.7°$. The thiocarbamate (**9**) had an $O_{4'}$-*endo*-$C_{4'}$-*exo* twist conformation, with the $C_{4'}$-*exo* twist conformation resulting in $C_{4'}$ 'below' the plane of the ring, this may cause the $O_{5'}$ to be directed 'away' from the ribose ring by approximately 180° (*trans*) and might also result in repulsive interactions between $O_{5'}$ and the $O_{4'}$, which is 'above' the plane (*endo*).

However, for the methyl-substituted oxazole nucleoside (**8**) the orientation about the $C_{4'}$–$C_{5'}$ bond ($\gamma = 60.8°$) can be described as having +*sc* (*gauche*) conformation, the preferred conformation for most pyrimidine nucleosides. This may well result from the twist sugar puckering, which allows the position of the $O_{5'}$ to be 'over' the ribose ring.

In summary, the conformational analysis for the above compounds can be tabulated as in Table 4.

4. Antiviral activity

Compounds **7**, **8**, **9** and **19** were evaluated for their inhibitory activity against a wide variety of viruses, including herpes simplex virus type 1 (HSV-1, strain KOS), HSV-2 (strain G), vaccinia virus, a thymidine kinase-deficient HSV-1 strain (KOS, ACV^R) and vesicular stomatitis virus (VSV) in human embryonic skin

muscle (E_6SM) cell cultures, VSV, Coxsackie virus B4 and respiratory syncytial virus in human HeLa cell cultures, parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4 and Punta Toro virus in simian Vero cell cultures and varicella-zoster virus (VZV, strains OKA and YS) and cytomegalovirus (strains AD169 and Davis) in human embryonic lung (HEL) cell cultures. None of the compounds showed inhibitory activity against any of the virus strains at 200 μg/mL. They were also not cytotoxic to the different cell lines (i.e., E_6SM, HEL, HeLa, Vero). The antiviral inactivity of the compounds can be most likely explained by a lack of substrate activity for cellular and/or viral nucleoside kinases for the compounds to be activated (phosphorylated), or, alternatively, by a lack of recognition of the compounds by the viral DNA or RNA polymerases in the (unlikely) case the compounds were to be converted intracellularly to their corresponding 5′-triphosphate derivatives.

5. Molecular modelling

From the MACROMODEL potential energy calculations, a general picture of the bicyclic molecules (**7**), (**8**) and (**9**) can be derived and it can be concluded that similar sugar puckering exists (*East* conformations) and all exist in the preferred *anti* conformation (Table 4). Although this conformation was unusually observed in the naturally occurring nucleosides, the possibility of this nucleoside adopting the unusual conformation could facilitate enzymatic phosphorylation.

It is of interest to note that in a total of 12 independent observations of the molecular conformation of the unsaturated nucleosides, all molecules having the dihydrofuran ring (d4 ring, as in stavudine (**2**)) had a nearly planar conformation with a slight tendency towards $O_{4'}$-*endo* (*East* conformation) and a preferred glycosidic angle range that is near to a high-*anti* conformation.[16] Thus, there is some degree of conformational similarity between the d4 pyrimidine nucleosides and the novel bicyclic nucleosides described here.

6. Experimental section

6.1. Materials and methods: chemistry

^{1}H and ^{13}C NMR spectra were recorded with a Brucker Avance DPX300 spectrometer operating at 300 and 75 MHz, with Me_4Si as internal standard. Mass spectra were determined by the EPSRC mass spectrometry centre (Swansea, UK). Microanalyses were determined by Medac Ltd (Surrey, UK). Flash column chromatography was performed with silica gel 60 (230–400 mesh) (Merck) and TLC was carried out on precoated silica plates (kiesel gel 60 F_{254}, BDH). Melting points were determined on an electrothermal instrument and are uncorrected. Infrared spectra were recorded using NaCl or KBr discs on a Perkin–Elmer 1600 series (FTIR) spectrometer.

6.1.1. 5′-*tert*-Butyldimethylsilyl-2′-amino-2′-deoxy-β-D-uridine (13) . To a solution of (**12**)[29] (1.10 g, 2.89 mmol) in anhydrous THF (30 mL) was added triphenyl phosphine (0.91 g, 3.47 mmol) and the reaction mixture stirred at room temperature until evolution of nitrogen ceased. Water (0.57 mL, 31.82 mmol) was added to the mixture, which was heated at 60 °C for 1.25 h. The reaction mixture was evaporated under reduced pressure and azeotroped with toluene to give the crude product as a pale yellow syrup. Purification by flash column chromatography (MeOH–$CHCl_3$, 7:93 v/v) gave the product as a white foam.

Yield: 0.89 g (87%), TLC system: MeOH–$CHCl_3$, 1:9 v/v, R_f: 0.54. IR (NaCl)/cm^{-1}: 3002–3180.3 (NH_2); 1691.6 (C=O). ^{1}H NMR ($CDCl_3$): δ 7.93 (d, $J_{6,5} = 8.1$ Hz, 1, H-6), 5.99 (d, $J_{1',2'} = 6.8$ Hz, 1, H-1′), 5.71 (d, $J_{5,6} = 8.1$ Hz, 1, H-5), 4.75 (br s, 3, NH_2, 3′-OH, ex), 4.24 (d, $J_{3',4'} = 5.2$ Hz, 1, H-3′), 4.17 (d, $J_{4',3'} = 5.3$ Hz, 1, H-4′), 3.94 (m, 1, H-5′), 3.86 (m, 1, H-5′), 3.49 (t, $J_{2',1'} = 6.5$, 1, H-2′), 0.95 (s, 9, $C(CH_3)_3$), 0.14 (s, 6, $Si(CH_3)_2$). ^{13}C NMR ($CDCl_3$): δ 164.31 (CO, C-4), 152.08 (CO, C-2), 140.49 (CH, C-6), 102.98 (CH, C-5), 90.09 (CH, C-1′), 86.98, 72.56 and 60.31 (CH, C-2′, C-3′ and C-4′), 64.12 (CH_2, C-5′), 26.33 (CH_3, $C(CH_3)_3$), 18.77 (C, $C(CH_3)_3$), −5.05 (CH_3, $Si(CH_3)_2$). Anal. for $C_{15}H_{27}O_5N_3Si{\cdot}0.8H_2O$ (371.89306); calcd C, 48.62%, H, 7.47%, N, 11.00%. Found: C, 48.45%, H, 7.75%, N, 11.30%.

6.1.2. 5′-*tert*-Butyldimethylsilyl-2′,3′-dideoxy-2′,3′-[3,4-*d*]oxazole-β-D-uridine (14). To a solution of (**13**) (0.87 g, 2.37 mmol) in dry DMF (25 mL) was added *N*,*N*-dimethylformamide dimethylacetal (DMFDMA) (0.65 mL, 4.91 mmol) and the reaction was stirred under nitrogen at room temperature for 20 h. The DMF was then evaporated under reduced pressure to give the crude product as a pale yellow syrup. The residue was left overnight to obtain the product. Purification by flash column chromatography (MeOH–$CHCl_3$, 2:98 v/v) with 1% Et_3N gave the product as a white foam.

Yield: 0.71 g (78%), TLC system: MeOH–$CHCl_3$, 1:4 v/v, R_f: 0.76. Mp: 163–164 °C. ^{1}H NMR ($CDCl_3$): δ 9.42 (s, 1, NH, ex), 7.59 (d, $J_{6,5} = 8.1$ Hz, 1, H-6), 7.06 (s, 1, CH=N), 5.87 (d, $J_{1',2'} = 2.8$ Hz, 1, H-1′), 5.82 (d, $J_{5,6} = 8.1$ Hz, 1, H-5), 5.18 (dd, $J_{3',4'} = 3.9$, $J_{3',2'} = 8.8$ Hz, 1, H-3′), 4.89 (m, 1, H-2′), 4.25 (d, $J_{4',3'} = 3.9$ Hz, 1, H-4′), 4.06 (dd, $J_{5',4'} = 3.9$, $J_{5'a,5'b} = 11.0$ Hz, 1, H-5′), 3.99 (dd, $J_{5',4'} = 4.3$, $J_{5'a,5'b} = 11.1$ Hz, 1, H-5′), 1.04 (s, 9, CH_3, $C(CH_3)_3$), 0.24 (s, 6, CH_3, $Si(CH_3)_2$). ^{13}C NMR ($CDCl_3$): δ 164.22 (CO, C-4), 156.08 (CH, CH=N), 150.63 (CO, C-2), 142.45 (CH, C-6), 102.98 (CH, C-5), 95.21 (CH, C-1′), 88.72, 82.51 and 77.21 (CH, C-2′, C-3′ and C-4′), 63.57 (CH_2, C-5′), 26.33 (CH_3, $C(CH_3)_3$), 18.84 (C, $C(CH_3)_3$), −4.95 (CH_3, $Si(CH_3)_2$). IR (KBr): 2928.3, 2854.3 (nonaromatic CH-str), 1700.1 (C=O), 1620.1 (C=N) cm^{-1}. LRMS (ES−) m/z: 366.0 (M−H); (ES+) m/z: 390.1 $(M+Na)^+$. Anal. for

$C_{16}H_{25}SiO_5N_3 \cdot 0.7H_2O$; calcd C, 50.56%, H, 6.63%, N, 11.05%. Found: C, 50.49%, H, 6.79%, N, 10.88%.

6.1.3. 2′,3′-Dideoxy-2′,3′-[3,4-*d*]oxazole-β-D-uridine (7). To compound (**14**) (0.70 g, 0.19 mmol) was added THF (3 mL) followed by TBAF (1 M solution in THF) (0.76 mL, 0.76 mmol) and the reaction stirred at room temperature for 1 h. The reaction mixture was evaporated under reduced pressure and the crude product was purified using flash column chromatography (MeOH–$CHCl_3$, 3:97 v/v) with 1% Et_3N to give the product as a colourless syrup.

Yield: 0.40 g (83%), TLC system: MeOH–$CHCl_3$, 1:9 v/v, R_f: 0.35, Mp: 122–124 °C. IR (NaCl)/cm^{-1}: 1692.2, 1680.3 (C=O) and 1626.9 (C=N). ^{1}H NMR (CD_3OD): δ 7.85 (d, $J_{6,5} = 8.0$ Hz, 1, H-6), 7.31 (d, $J = 1.7$ Hz, 1, CH=N), 5.84 (d, $J_{1',2'} = 3.0$ Hz, 1, H-1′), 5.79 (d, $J_{5,6} = 8.0$ Hz, 1, H-5), 5.22 (dd, $J_{3',4'} = 4.3$, $J_{3',2'} = 8.9$ Hz, 1, H-3′), 5.02 (m, 1, H-2′), 4.17 (q, $J_{3',4'} = 4.3$ Hz, 1, H-4′), 3.94 (dd, $J_{5',4'} = 4.2$, $J_{5'a,5'b} = 11.9$ Hz, 1, H-5′), 3.88 (dd, $J_{5',4'} = 4.2$, $J_{5'a,5'b} = 11.9$ Hz, 1, H-5′). ^{13}C NMR (CD_3OD): δ 166.59 (CO, C-4), 158.44 (CH, CH=N), 152.30 (CO, C-2), 144.68 (CH, C-6), 103.21 (CH, C-5), 95.99 (CH, C-1′), 89.79, 83.84 and 77.63 (CH, C-2′, C-3′ and C-4′), 63.21 (CH_2, C-5′). HRMS (ES+) for $C_{10}H_{11}N_3O_5$ $(M+H)^+$: calcd 254.077; fund, 254.0781.

6.1.4. 5′-*tert*-Butyldimethylsilyl-2′,3′-dideoxy-2′,3′-[3,4-*d*]methyl-oxazole)-β-D-uridine (15). To compound (**13**) (0.14 g, 0.39 mmol) dissolved in dry DMF (5 mL) was added *N*,*N*-dimethylacetamide dimethylacetal (DMADMA) (0.12 mL, 0.783 mmol) slowly and the reaction mixture was stirred under nitrogen at room temperature for 4 h and 15 min. Then, DMF was evaporated under reduced pressure. The crude product was purified by flash column chromatography (eluted with methanol–chloroform, 3:97 v/v) with 1% Et_3N to give the product as a colourless syrup.

Yield: 90 mg (60%), TLC system: MeOH–$CHCl_3$, 15:85 v/v, R_f: 0.52. Mp: 216–217 °C ^{1}H NMR ($CDCl_3$): 9.90 (br s, 1, NH, ex), 7.61 (d, $J_{6,5} = 8.1$ Hz, 1, H-6), 5.91 (d, $J_{1',2'} = 2.7$ Hz, 1, H-1′), 5.81 (d, $J_{5,6} = 8.1$ Hz, 1, H-5), 5.16 (dd, $J_{3',4'} = 4.0$, $J_{3',2'} = 8.7$ Hz, 1, H-3′), 4.85 (dd, $J_{2',1'} = 2.6$, $J_{2',3'} = 8.7$ Hz, 1, H-2′), 4.24 (d, $J_{4',3'} = 4.0$ Hz, 1, H-4′), 4.06 (dd, $J_{5',4'} = 3.8$, $J_{5'a,5'b} = 11.1$ Hz, 1, H-5′), 3.98 (dd, $J_{5',4'} = 4.0$, $J_{5'a,5'b} = 11.1$ Hz, 1, H-5′), 2.19 (s, 3, CH_3), 1.02 (s, 9, CH_3, $C(CH_3)_3$), 0.60 (s, 6, CH_3, $Si(CH_3)_2$). ^{13}C NMR ($CDCl_3$): δ 166.83 (CO, C-4), 163.94 (C, C=N), 150.52 (CO, C-2), 142.29 (CH, C-6), 102.86 (CH, C-5), 95.07 (CH, C-1′), 88.68, 83.24 and 73.22 (CH, C-2′, C-3′ and C-4′), 63.63 (CH_2, C-5′), 26.28 (CH_3, $C(CH_3)_3$), 18.76 (C, $C(CH_3)_3$),14.31 (CH_3) −4.93 (CH_3, $Si(CH_3)_2$). LRMS (ES+) m/z: 403.9 $(M+Na)^+$, 784.7 $(2M+Na)^+$. Anal. for $C_{17}H_{27}SiO_5N_3$; calcd C, 53.52%, H, 7.13%, N, 11.01%. Found C, 53.18%, H, 7.20%, N, 10.86%.

6.1.5. 2′,3′-Dideoxy-2′,3′-[3,4-*d*](2-methyl-oxazole)-β-D-uridine (8). To compound (**15**) (0.25 g, 0.65 mmol) was added THF (5 mL) followed by TBAF (1 M solution in THF) (2.62 mL, 2.62 mmol) and the reaction stirred at room temperature for 30 min. The reaction mixture was evaporated under reduced pressure and the crude product was purified using flash column chromatography (MeOH–$CHCl_3$, 2:98 v/v) with 1% Et_3N to give the product as a white solid.

Yield: 0.15 g (86%), TLC system: MeOH–$CHCl_3$, 1:9 v/v, R_f: 0.32. Mp: 122–124 °C. ^{1}H NMR ($CDCl_3$): δ 7.38 (d, $J_{6,5} = 8.0$ Hz, 1, H-6), 6.40 (br s, 2, H-5′-OH, NH, ex), 5.63 (d, $J_{5,6} = 7.9$ Hz, 1, H-5), 5.51 (d, $J_{1',2'} = 2.6$ Hz, 1, H-1′), 5.13 (dd, $J_{3',4'} = 4.3$, $J_{3',2'} = 8.8$ Hz, 1, H-3′), 4.82 (m, 1, H-2′), 4.03 (m, 1, H-4′), 3.86 (dd, $J_{5',4'} = 4.0$, $J_{5'a,5'b} = 11.9$ Hz, 1, H-5′), 3.77 (dd, $J_{5',4'} = 4.0$, $J_{5'a,5'b} = 11.9$ Hz, 1, H-5′), 2.00 (s, 3, CH_3). ^{13}C NMR ($CDCl_3$): δ 167.16 (CO, C-4), 164.69 (C, C=N), 151.29 (CO, C-2), 143.65 (CH, C-6), 102.96 (CH, C-5), 96.88 (CH, C-1′), 88.75, 82.86 and 77.45 (CH, C-2′, C-3′ and C-4′), 62.41 (CH_2, C-5′), 14.31 (CH_3). HRMS (ES+) for $C_{11}H_{14}N_3O_5$ $(M+H)^+$: calcd 268.0933; found, 268.0932.

6.1.6. 5′-*tert*-Butyldimethylsilyl-2′,3′-dideoxy-2′,3′-[3,4-*d*](oxazolidin-2-thione)-β-D-uridine (16). A solution of 1, 1′-thiocarbonyldiimidazole (86 mg of 90% reagent, 0.43 mmol) in anhydrous dichloromethane (2.50 mL) was added dropwise to a suspension of (**13**) (155 mg, 0.43 mmol) in dichloromethane (2.50 mL) and the reaction mixture stirred under nitrogen at room temperature for 4.5 h. The reaction mixture was diluted with dichloromethane (80 mL) and washed with water (2×50 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to give crude product, which was purified by flash column chromatography (MeOH–CH_2Cl_2, 15:98.5 v/v) to give a white solid.

Yield: 0.16 g (94%), TLC system: MeOH–$CHCl_3$, 1:9 v/v, R_f: 0.62. Mp: 230–234 °C. ^{1}H NMR ($CDCl_3$): 10.83 (s, 1, NH, ex), 10.37 (s, 1, NH, ex), 7.53 (d, $J_{6,5} = 8.2$ Hz, 1, H-6), 5.63 (d, $J_{5,6} = 8.2$ Hz, 1, H-5), 5.58 (d, $J_{1',2'} = 2.6$ Hz, 1, H-1′), 5.26 (dd, $J_{3',4'} = 1.6$, $J_{3',2'} = 8.2$ Hz, 1, H-3′), 4.78 (d, $J_{4',3'} = 1.5$ Hz, 1, H-4′), 4.49 (dd, $J_{2',1'} = 2.6$, $J_{2',3'} = 8.2$ Hz, 1, H-2′), 3.94 (dd,$J_{5',4'} = 2.0$, $J_{5'a,5'b} = 11.8$ Hz, 1, H-5′), 3.83 (dd, $J_{5',4'} = 2.1$, $J_{5'a,5'b} = 11.8$ Hz, 1, H-5′), 0.8 (s, 9, $C(CH_3)_3$), 0.00 (s, 6, $Si(CH_3)_2$). ^{13}C NMR ($CDCl_3$): δ 189.19 (C=S), 162.79 (CO, C-4), 152.57 (CO, C-2), 138.89 (CH, C-6), 103.00 (CH, C-5), 96.15 (CH, C-1′), 88.81, 87.09 and 70.06 (CH, C-2′, C-3′ and C-4′), 63.82 (CH_2, C-5′), 26.18 (CH_3, $C(CH_3)_3$), 18.61 (C, $C(CH_3)_3$), −5.02, −5.19 (CH_3, $Si(CH_3)_2$). IR (KBr): 3500–2800 (imine N–H broad), 3174.0 (aromatic CH-str), 2928.6, 2858.1 (nonaromatic CH-str), 1713.4, 1681.7 (C=O), 1259.9 (C=S) cm^{-1}. LRMS (ES+) m/z: 421.9 $(M+Na)^+$, 400 $(M+H)^+$. Anal. for $C_{16}H_{25}SSiO_5N_3$; calcd C, 48.10%, H, 6.31 %, N, 10.51%. Found: C, 47.94%, H, 6.36%, N, 10.42%.

6.1.7. 2′,3′-Dideoxy-2′,3′-[3,4-*d*](oxazolidin-2-thione)-β-D-uridine (9) and 2′,3′-dideoxy-2′,3′-[3,4-*d*](4,5-dihydro-oxazole-2-thiol)-β-D-uridine (17). To compound (**16**) (0.12 g, 0.30 mmol) was added THF (2 mL) followed by

TBAF (1 M solution in THF) (0.60 mL, 0.60 mmol) and the reaction stirred at room temperature for 8 h. The reaction mixture was evaporated under reduced pressure and the crude product was purified using flash column chromatography (MeOH–$CHCl_3$, 5:95 v/v) to give the product as a white solid.

Yield: 0.06 g (72 %) containing approx. 12% of the minor isomer as judged by NMR. TLC system: MeOH–$CHCl_3$, 1:9 v/v, R_f: 0.3. Mp: 244–250 °C (decomp.).

Major tautomer (**9**)—^{1}H NMR (DMSO-d_6): δ 11.49 (s, 1, NH, ex), 10.53 (s, 1, NH, ex), 7.71 (d, $J_{6,5} = 8.1$ Hz, 1, H-6), 5.79 (d, $J_{1',2'} = 3.0$ Hz, 1, H-1′), 5.68 (d, $J_{5,6} = 8.1$ Hz, 1, H-5), 5.35 (dd, $J_{3',4'} = 4.0$, $J_{3',2'} = 8.6$ Hz, 1, H-3′), 5.19 (t, $J_{5'OH,5'} = 5.5$ Hz, 1, 5′-OH, ex), 4.80 (dd, $J_{2',1'} = 3.0$, $J_{2',3'} = 8.6$ Hz, 1, H-2′), 4.17 (t, $J_{4',3'} = 4.0$ Hz, 1, H-4′), 3.64 (m, 2, H-5′) ^{13}C NMR (DMSO-d_6): δ 185.57 (C, C=S), 161.68 (CO, C-4), 148.40 (CO, C-2), 142.60 (CH, C-6), 102.05 (CH, C-5), 92.87, 86.67, 86.15 and 65.56 (CH, C-1′, C-2′, C-3′ and C-4′), 63.41 (CH_2, C-5′).

Minor tautomer (**17**)—^{1}H NMR (DMSO-d_6): δ 7.78 (d, $J_{6,5} = 8.1$ Hz, 1, H-6), 7.30 (s, 1, NH, ex), 5.76 (s, 1, SH), 5.74 (d, $J_{1',2'} = 3.0$ Hz, 1, H-1′), 5.68 (d, $J_{5,6} = 8.1$ Hz, 1, H-5), 5.15 (t, $J_{5'OH,5'} = 5.5$ Hz, 1, 5′-OH, ex), 5.00 (dd, $J_{3',4'} = 4.4$, $J_{3',2'} = 8.9$ Hz, 1, H-3′), 4.12 (m, 1, H-2′), 3.90 (q, $J_{4',3'} = 4.6$ Hz, 1, H-4′), 3.64 (m, 2, H-5′). ^{13}C NMR (DMSO-d_6): δ 206.02 (C, CSH), 161.72 (CO, C-4), 149.12 (CO, C-2), 139.13 (CH, C-6), 100.55 (CH, C-5), 85.09, 87.09, 70.73, 53.44 (CH, C-1′, C-2′, C-3′ and C-4′), 61.69 (CH_2, C-5′). LRMS (CI) m/z: 286.1 $(M+H)^+$; (ES+) m/z: 308.0 $(M+Na)^+$. HRMS (ES+) for $C_{10}H_{12}N_3O_5S$ $(M+H)^+$: calcd 286.0497; found, 286.0496.

6.1.8. 5′-*tert*-Butyldimethylsilyl-2′,3′-dideoxy-2′,3′-[3,4-*d*](oxazolidin-2-thione)-β-D-4-thiouridine (18) and 2′,3′-dideoxy-2′,3′-[3,4-*d*](oxazolidin-2-thione)-β-D-4-thiouridine (19). To a suspension of (**14**) (0.1 g, 0.25 mmol) in anhydrous toluene (0.9 mL) was added Lawesson's reagent (0.12 g, 0.3 mmol) and the reaction heated at 80 °C for 4 h. The reaction mixture was co-evaporated with methanol to give the crude product as a yellow solid. Purification by flash column chromatography (CH_2Cl_2–EtOAc, 2.5:97.5 v/v) gave (**18**) as a yellow solid.

Yield: 0.035 g (34%), TLC system: EtOAc–CH_2Cl_2, 1:1 v/v, R_f: 0.75. Mp: 142–144 °C. ^{1}H NMR (DMSO-d_6): δ 12.86 (s, 1, NH, ex), 10.60 (s, 1, NH, ex), 7.59 (d, $J_{6,5} = 7.3$ Hz, 1, H-6), 6.30 (d, $J_{5,6} = 7.4$ Hz, 1, H-5), 5.71 (d, $J_{1',2'} = 1.8$ Hz, 1, H-1′), 5.33 (dd, $J_{3',4'} = 2.9$, $J_{3',2'} = 8.2$ Hz, 1, H-3′), 4.82 (d, $J_{2',3'} = 8.2$ Hz, 1, H-4′), 4.38 (m, 1, H-4′), 3.87 (m, 2, H-5′, H-5″), 0.87 (s, 9, $C(CH_3)_3$), 0.06 (s, 6, $Si(CH_3)_2$). ^{13}C NMR ($CDCl_3$): δ 190.88 (C=S, C-4), 187.79 (C=S, carbamate), 147.97 (CO, C-2), 137.40 (CH, C-6), 112.83 (CH, C-5), 94.27, 87.35, 86.34 and 66.26 (CH, C-1′, C-2′, C-3′ and C-4′), 63.04 (CH_2, C-5′), 26.07 (CH_3, $C(CH_3)_3$), 18.76 (C, $C(CH_3)_3$), −5.03 ($2 \times CH_3$, $Si(CH_3)_2$). LRMS (CI) m/z: 416.2 $(M+H)^+$.

Further elution (CH_2Cl_2–EtOAc, 7:93 v/v) gave the deprotected product (**19**) as a yellow solid.

Yield: 0.05 g (66%). TLC system: EtOAc–CH_2Cl_2, 1:1 v/v, R_f: 0.29. Mp: 142–146 °C. ^{1}H NMR (DMSO-d_6): δ 12.86 (s, 1, NH, ex), 10.56 (s, 1, NH, ex), 7.68 (d, $J_{6,5} = 7.5$ Hz, 1, H-6), 6.34 (d, $J_{5,6} = 8.1$ Hz, 1, H-5) 5.76 (d, $J_{1',2'} = 2.6$ Hz, 1, H-1′), 5.36 (dd, $J_{3',4'} = 3.6$, $J_{3',2'} = 8.4$ Hz, 1, H-3′), 5.23 (t, $J_{5'OH,5'} = 5.1$ Hz, 1, 5′-OH, ex), 4.85 (dd, $J_{2',1'} = 2.6$, $J_{2',3'} = 8.4$ Hz, 1, H-2′), 4.27 (dd, $J_{5',4'} = 4.7$ Hz, $J_{5',5''} = 8.6$ Hz, 1, H-5′), 3.66 (m, 2, H-4′, H-5″). ^{13}C NMR (DMSO-d_6): δ 190.93 (C=S, C-4), 187.79 (C, C=S), 147.92 (CO, C-2), 137.49 (CH, C-6), 112.87 (CH, C-5), 93.47, 87.34, 86.12 and 65.93 (CH, C-1′, C-2′, C-3′ and C-4′), 61.06 (CH_2, C-5′). IR (KBr): 3483.3 (NH), 3500–2800 (imine NH and OH, broad), 3127.4 (aromatic CH str), 1693.7 (C=O), 1613.9 (C–N), 1280 (C=S) cm^{-1}. LRMS (EI) m/z: 301.0 $(M)^+$; (ES−) m/z: 300.0 $(M-H)^+$. HRMS (EI/CI) for $C_{10}H_{12}N_3O_4S_2$ $(M+H)^+$: calcd 302.0264; found, 302.0265 $(M-H)^+$.

6.2. Materials and experimental procedures: virology

The antiviral assays were based on an inhibition of virus-induced cytopathicity in either E_6SM, HeLa, Vero or HEL cell cultures, following previously established procedures.[36–40] Briefly, confluent cell cultures in microtiter trays were inoculated with 100 $CCID_{50}$ of virus, 1 $CCID_{50}$ being the virus dose required to infect 50% of the cell cultures. After a 1 h virus adsorption period, residual virus was removed and the cell cultures were incubated in the presence of varying concentrations (400, 200, 100,... μg/mL) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures.

Following viruses were included in the study: herpes simplex virus type 1 (HSV-1 strain KOS), HSV-2 (strain G), a thymidine kinase (TK)-deficient HSV-1 strain (HSV-1/TK$^-$ ACV^R), vaccinia virus and vesicular stomatitis virus (VSV) in E_6SM cell cultures, cytomegalovirus (strain AD169 and Davis), varicella-zoster virus (strains YS and OKA) and TK-deficient VZV (strains 07/1 and YS/R) in HEL cell cultures, vesicular stomatitis virus, Coxsackie virus B4 and respiratory syncytial virus (RSV) in HeLa cell cultures, and parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4 and Punta Toro virus in Vero cell cultures.

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