6-Azapyrimidine-2′-deoxy-4′-thionucleosides: Antiviral Agents against TK+ and TK− HSV and VZV Strains

Hannah L. Maslen,[†] David Hughes,[‡] Mike Hursthouse,[‡] Erik De Clercq,[§] Jan Balzarini,[§] and Claire Simons*,[†]

Medicinal Chemistry Division, Welsh School of Pharmacy, Cardiff University, King Edward VII Avenue, Cardiff CF10 3XF, UK; EPSRC X-ray Crystallography Service, Department of Chemistry, University of Southampton, Highfield, Southampton SO17 1BJ, UK; and Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium

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The synthesis of a series of novel 1-(2-deoxy-4-thio-β-D-*erythro*-pentofuranosyl)-(6-azapyrimidine) nucleosides is described. X-ray crystallographic data of the thymidine derivative allowed conformational analysis, which indicated a twist ($^3T_2$) sugar conformation. Hydrogen-bonded assemblies for the crystal structure were determined using PLATON software to allow further interpretation of the crystal packing and base interactions. The 6-azapyrimidine nucleosides described were evaluated against a range of viral strains. The thymidine analogue showed pronounced activity against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), varicella-zoster virus (VZV), and vaccinia virus. This compound lost only 5- to 10-fold of its antiviral activity against thymidine kinase (TK)-deficient HSV-1 and VZV strains. These observations suggest that the compounds may not entirely depend on viral TK-catalyzed phosphorylation for antiviral activity and/or use an alternative metabolic activation pathway, and/or display a unique mechanism of antiviral action by the unmetabolized nucleoside analogue.

Introduction

Both 5- and 6-azapyrimidine nucleosides are of interest owing to their biological properties (Figure 1).[1] 5-Azacytidine **1** is active against T-4 lymphomas and L1210 leukaemia. As a result, 5-azacytidine and its 2′-deoxy derivative **2** are used clinically in the treatment of acute leukaemia.[2,3] 5,6-Dihydro-5-azathymidine **3**, a naturally occurring nucleoside antibiotic isolated from *Streptomyces platensis* var. *clarensis*, exhibits in vitro activity against DNA viruses.[4] In the 6-azapyrimidine nucleoside series, 6-azauridine monophosphate **4** is of most interest, owing to its antiviral activity which results from inhibition of orotidine 5′-monophosphate decarboxylase, an enzyme involved in the de novo pyrimidine mononucleotide biosynthesis.[5]

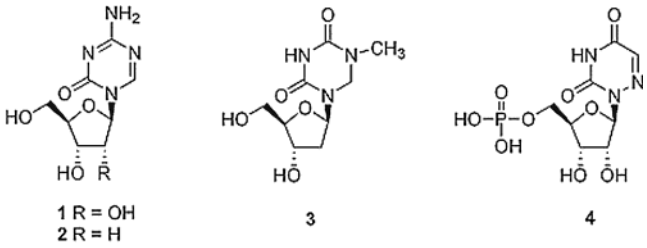

Figure 1. Biologically active azapyrimidines.

As with all conventional glycosidic linkages, these azapyrimidine nucleosides are susceptible to cleavage by nucleoside phosphorylases. The toxicity associated with these azapyrimidine nucleosides may well result from release of the 5- or 6-azapyrimidine base, which are known to display narcotic activity and cause central nervous system disturbances in man.[6] This toxicity detracts from the potential of the azapyrimidine nucleosides; however, the demonstrated resistance of 4′-thionucleosides toward phosphorolytic cleavage[7] may provide a less toxic derivative with clinical potential.

1-(2-Deoxy-4-thio-β-D-*erythro*-pentofuranosyl)-(6-azauracil), which has previously been described,[8] was devoid of antiviral activity. The lack of anti-herpes activity was proposed to be owing to poor affinity for the HSV-1 thymidine kinase. 5-Substituted pyrimidine nucleosides often display potent anti-herpes virus activity;[9,10] therefore, it was of interest to prepare 1-(2-deoxy-4-thio-β-D-*erythro*-pentofuranosyl)-(5-substituted-6-azapyrimidine) nucleosides (**5**β−**7**β, **8**, and **9**) for biological evaluation (Figure 2).

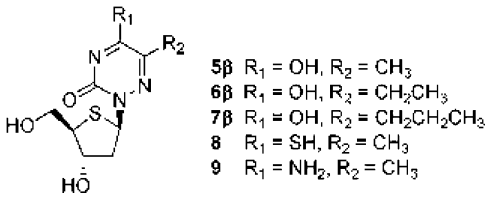

Figure 2. Structures of 6-azapyrimidine 2′-deoxy-4′-thionucleosides **5**β−**7**β, **8**, and **9**.

Results

Chemistry. The 5-methyl-6-azapyrimidine **10** was commercially available; the 5-ethyl- and 5-propyl-6-azapyrimidines, **16** and **17** respectively, were prepared via the semicarbazones, **14** and **15**, by the reaction of semicarbazide hydrochloride **11** with the corresponding 2-keto-carboxylic acids **12** and **13**, according to a literature procedure (Scheme 1).[6] A 25 °C discrepancy be-

* To whom correspondence should be addressed. Tel: +44 (0)2920 876307. Fax: +44 (0)2920 874149. E-mail: SimonsC@Cardiff.ac.uk.
† Cardiff University.
‡ University of Southampton.
§ Katholieke Universiteit Leuven.

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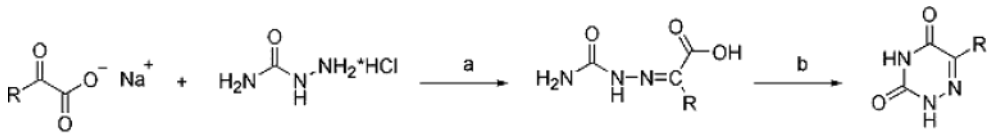
Scheme 1[a]



12 R = Et
13 R = nPr

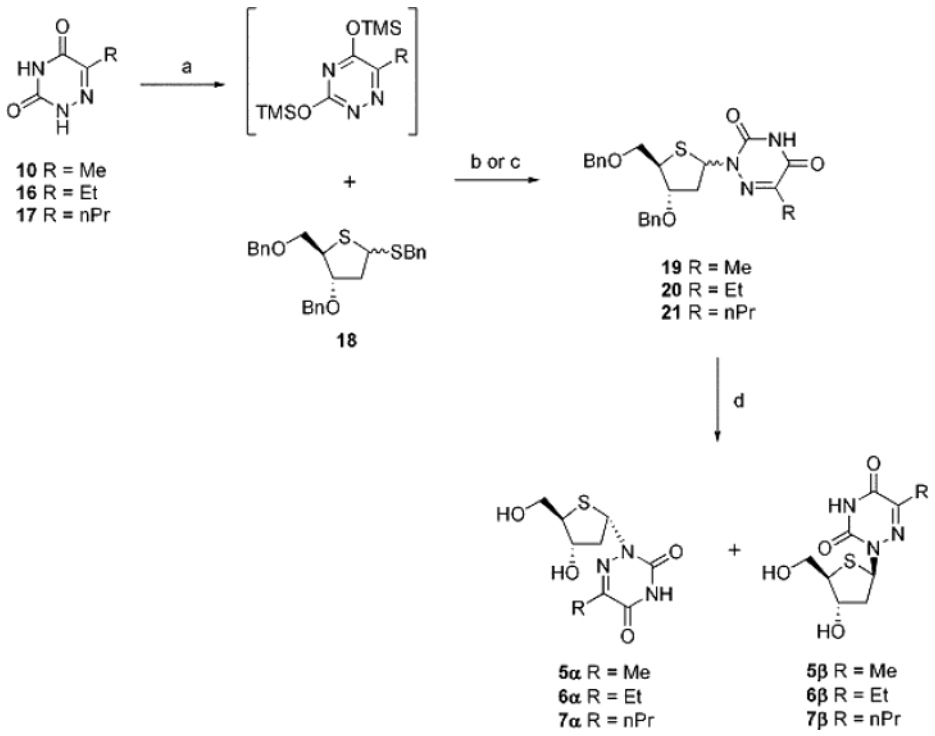
11

14 R = Et
15 R = nPr

16 R = Et
17 R = nPr

[a] Conditions: (a) R = Et, H_2O, 1 h; R = Pr, H_2O, EtOH, 100 °C, 1 h then 4 °C, 12 h. (b) i. NaOEt, EtOH, ethylene glycol, 100 °C, 48−72 h; ii. aqueous HCl (pH 2), 4 °C, 12 h.

Scheme 2[a]



10 R = Me
16 R = Et
17 R = nPr

18

19 R = Me
20 R = Et
21 R = nPr

5α R = Me
6α R = Et
7α R = nPr

5β R = Me
6β R = Et
7β R = nPr

[a] Conditions: (a) *N*,*O*-Bis(trimethylsilyl)acetamide, CH_3CN, 1 h. (b) ICl, CH_3CN, 4 Å molecular sieves, 24 h. (c) NIS, CH_3CN, 4 Å molecular sieves, 24 h. (d) BCl_3, CH_2Cl_2, −80 °C, 4 h.

tween the literature mp (175−176 °C) and the mp of the prepared compound **15** (200−202 °C) was noted; however, ^1H and ^{13}C NMR confirmed the purity and structure of the prepared compound. ^{13}C NMR displayed characteristic signals for the carboxylic acid, $\delta \sim 165$, amide, $\delta \sim 157$, and N═C, $\delta \sim 140$. Cyclization of the semicarbazones **14** and **15** using sodium ethoxide gave the required 5-alkyl-6-azapyrimidines **16** and **17** in low yields and, in the case of **17**, the reaction failed to reach completion even after 72 h reaction time with a 1:5 mixture of **13**:**17** obtained (Scheme 1).

The benzyl-protected 2′-deoxy-4′-thionucleosides **19**−**21** were prepared by the reaction of the thiosugar **18**[11] with the bis-silylated 6-azapyrimidine bases, prepared by the reaction of **10**, **16**, and **17** with *N*,*O*-bis(trimethylsilyl)acetamide in anhydrous acetonitrile for 1 h, in the presence of either iodine monochloride or *N*-iodosuccinimide (NIS) (Scheme 2). The use of iodine reagents as coupling catalysts was based on the procedure described by Kim and Misco[12] for the preparation of 4′-thionucleosides (Sugiyama coupling method), which results in approximately 1:1 ratios of the α- and β-anomers in reasonable yields (Table 1). Debenzylation of **19**−**21** with boron trichloride allowed the separation of the anomers (Scheme 2).

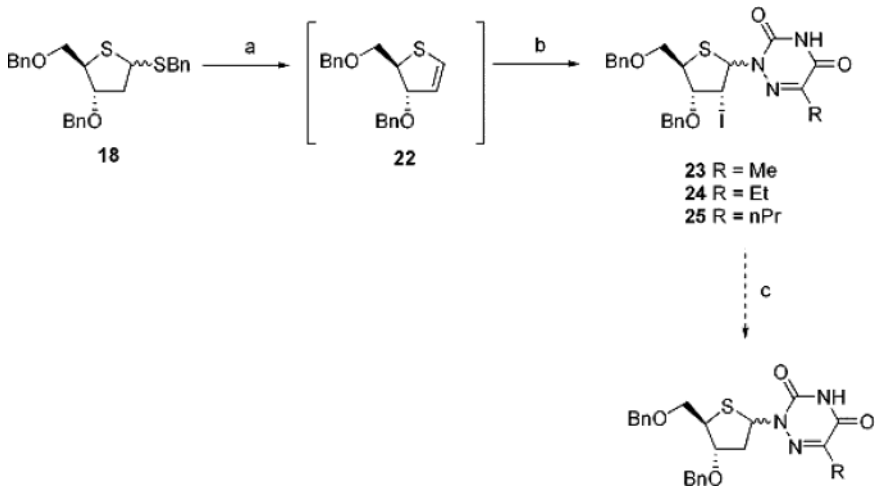
Table 1. α:β Ratios from Sugiyama Coupling

compound	α:β ratio	yield, %
19, R = Me	1:1	78
20, R = Et	1:1	70
21, R = nPr	1:1	76

In an attempt to improve the anomeric ratio of the coupled nucleosides, the glycal methodology was applied.[13] This involved initial reaction of the thiosugar **18** with iodine monochloride in the presence of 2,6-di-*tert*-butyl-4-methylpyridine at 0 °C for 5 h to form the glycal **22** (Scheme 3). Introduction of the silyl protected azapyrimidine bases and an additional quantity of iodine monochloride resulted in formation of the 2-iodonucleosides **23**−**25** in reasonable yields (Scheme 3). As can be seen from Table 2, improved α:β ratios were obtained; however, this promising ratio was negated by the unreliability of the radical deiodination step which, in our hands, resulted in complex mixtures. Owing to the unsatisfactory deiodination reaction, the Sugiyama method (Scheme 2) would appear to provide the optimum coupling procedure for these nucleosides.

Further modification of the base moiety involved preparation of the C-4 thiocarbonyl derivative **8**, which was subsequently converted to the 6-aza-5-methyl-

Scheme 3[a]



[a] Conditions: (a) ICl, CH_2Cl_2, 2,6-di-*tert*-butyl-4-methylpyridine, 0 °C, 5 h. (b) i. (6-azapyrimidine)TMS_2, CH_2Cl_2; ii. ICl, CH_2Cl_2, 16 h. (c) azobiscyclohexane carbonitrile, Bu_3SnH, toluene, 45−60 °C.

Table 2. α:*β* Ratios from Glycal Coupling

compound	α:*β* ratio	yield, %
23, R = Me	1:4	70
24, R = Et	1:3	67
25, R = nPr	1:3	67

Table 3. ^{1}H NMR Data for Compounds **5**−**9**

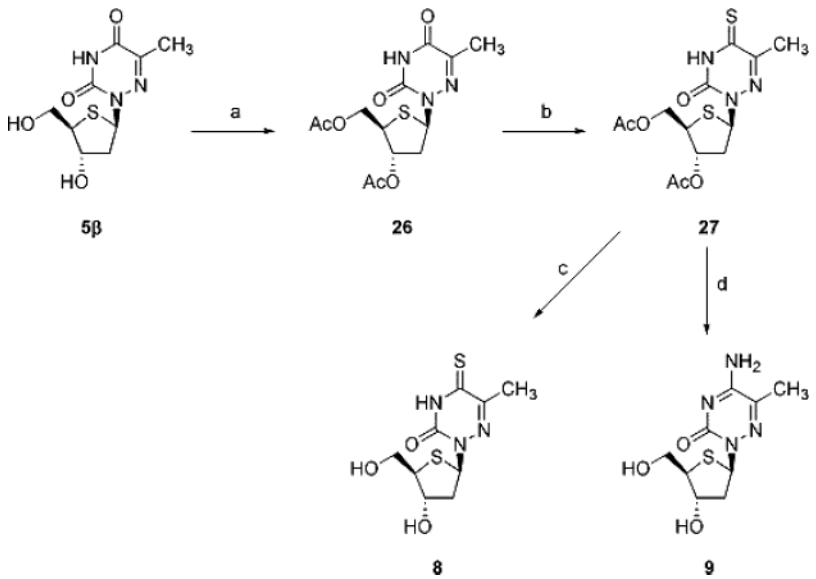
compound	H-1′	$J_{1',2'}$	H-2′ endo	H-2′ exo	$\Delta_{2'endo,2'exo}$
5α[a]	6.34, dd	5.9, 8.1	2.65, m	2.51, m	0.14
5*β*[a]	6.37, dd	5.1, 7.3	2.70, m	2.35, m	0.35
6α[b]	6.13, t	7.7	2.49, m[c]		-
6*β*[b]	6.19, *ψ*t	5.5, 6.8	2.50, m[c]	2.17, m	0.33
7α[a]	6.13, dd	5.9, 8.2	2.73, m[c]	2.61, m	0.12
7*β*[a]	6.30, dd	4.7, 7.3	2.57, m[c]	2.29, m	0.28
8[a]	6.29, dd	5.0, 7.3	2.69, m	2.37, m	0.32
9[b]	6.21, *ψ*t	6.0, 6.7	2.46, m	2.11, m	0.35

[a] CD_3OD. [b] DMSO-d_6. [c] Overlapping with CH_2 from Et/Pr signal.

cytosine nucleoside **9** using conventional methodology (Scheme 4). Preparation of **8** required initial acetyl protection of 1-(2-deoxy-4-thio-*β*-D-*erythro*-pentofuranosyl)-(6-azathymine) **5***β*. The acetylated nucleoside **26** was then treated with Lawesson's reagent[14] to give **27**. Reaction of **27** with methanolic ammonia at 0 °C and then increasing to room temperature gave the deacetylated C-4 thiocarbonyl nucleoside **8**. Reaction of **27** with methanolic ammonia at 30 °C in a sealed vessel for 72 h gave the deacetylated C-4 amino nucleoside **9** (Scheme 4).

Confirmation of anomeric configuration was obtained by ^{1}H NMR (Table 3) and, in the case of **5**, by X-ray crystallography (Figure 2). It was possible to distinguish between the anomers from the chemical shift of H-1′ with H-1′*β* occurring further downfield than H-1′α. The H-2′ signal splitting patterns were very distinctive with the two multiplets for H-2′α (endo and exo) separated by approximately 0.1 ppm, whereas the two multiplets for H-2′*β* were separated by approximately 0.3 ppm. This characteristic H-2′ splitting pattern has previously been observed for 2′-deoxy-4′-thio-nucleosides.[8,15]

Scheme 4[a]



[a] Conditions: (a) Ac_2O, pyridine, 16 h. (b) Lawesson's reagent, toluene, reflux, 3 h. (c) NH_3/MeOH, 0 °C, 4 h then room temperature, 4 h. (d) NH_3/MeOH, 30 °C, sealed vessel, 72 h.

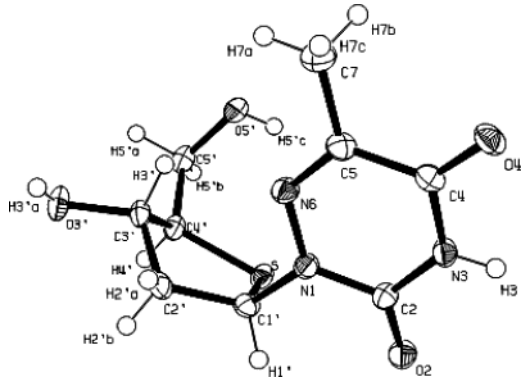


Figure 3. ORTEP diagram of **5**β.

Table 4. Selected Geometrical Parameters for Compound **5**β Compared with 2′-Deoxy-4′-thiothymidine

	5β	4′S-T (II)[17]
Bond Lengths (Å)		
C1′−C2′	1.516 (2)	1.525 (4)
C2′−C3′	1.523 (2)	1.508 (4)
C3′−C4′	1.527 (2)	1.545 (4)
C1′−S4′	1.838 (15)	1.839 (3)
C4′−S4′	1.825 (16)	1.831 (3)
C1′−N1	1.469 (19)	1.480 (4)
Bond Angles (deg)		
C1′C2′C3′	108.41 (13)	106.2 (3)
C2′C3′C4′	107.63 (13)	106.6 (2)
C3′C4′S4′	106.12 (11)	105.4 (2)
C4′S4′C1′	94.87 (7)	94.0 (1)
S4′C1′C2′	106.24 (11)	105.9 (2)
S4′C1′N1	111.00 (10)	113.5 (2)
Torsion Angles (deg)		
C4′S4′C1′C2′ (v_0)	10.37 (11)	−14.5 (2)
S4′C1′C2′C3′ (v_1)	−32.52 (14)	39.0 (3)
C1′C2′C3′C4′ (v_2)	44.58 (16)	−50.4 (3)
C2′C3′C4′S4′ (v_3)	−35.03 (15)	37.9 (3)
C3′C4′S4′C1′ (v_4)	14.11 (12)	−13.0 (3)
O5′C5′C4′C3′ (γ)	65.34 (17)	179.0 (2)
C5′C4′C3′O3′ (δ)	78.39 (15)	-
S4′C1′N1C2 (χ)	−76.04 (15)	−144.2 (3)
S4′C1′N1N6	89.64 (14)	-
Pseudorotational Parameters (deg)		
phase angle (P)	2.608	177.7
degree of pucker (v_m)	44.626	47.9
sugar conformation	$^3T_2$	^2E
glycosidic conformation	anti	anti
C4′−C5′ conformation	gauche g+ (+sc)	trans (ap)

Conformational Analysis. X-ray crystallography provided absolute confirmation of the anomeric configuration of the 6-aza-thymidine nucleosides **5**β (Figure 3).

Selected geometrical parameters[16] for **5**β are described in Table 4, and compared with 2′-deoxy-4′-thiothymidine (4′S-T),[17] a known substrate for viral thymidine kinase which displays activity against herpes simplex virus (HSV).[18]

The X-ray crystallographic data indicated an anti conformation (torsional angle $\chi = -76.04°$). The major differences between **5**β and 4′S-T were the conformation about the C4′−C5′ bond, with a gauche (g+ or +sc) orientation for **5**β compared with trans (or ap) orientation for 4′S-T, and the ring puckering with **5**β displaying a North-type ($^3T_2$) and 4′S-T a South-type (^2E) conformation.

Antiviral Activity. The antiviral effects of compounds **5**β, **6**β, **7**, **8**, **9**, and **27** were evaluated against cytomegalovirus (strains AD169 and Davis) and varicella-zoster virus (strains YS and OKA) as well as the thymidine kinase (TK) deficient 07/1 and YS/R VZV strains (Table 5). Compound **5**β displayed activity comparable with acyclovir (ACV) ($EC_{50} \sim 1$ μM); however, it was considerably less active than brivudin ((*E*)-5-(2-bromovinyl)-2′-deoxyuridine, BVDU) (EC_{50}: 0.003 μM). However, importantly **5**β showed a reasonably good retention of antiviral activity in VZV/TK$^-$ strains, whereas BVDU lost activity against TK$^-$ VZV strains by at least 4 orders of magnitude. It should also be mentioned that **5**β was inhibitory to both HSV-1 and HSV-2, whereas BVDU selectively inhibited HSV-1, but not HSV-2.

Compound **5**β did not inhibit CMV replication in cell culture at 200 μM. The other compounds, **6**β, **7**β, **8**, **9**, and **27**, were also devoid of anti-VZV/CMV activity at 40 μM.

Compound **5**β was also found to display pronounced activity against HSV-1 (strain KOS) and HSV-2 (strain G), with comparable EC_{50} values (1.3−3.2 μg/mL) (Table 6). However, the antiviral potential of these compounds were inferior to those of acyclovir and BVDU. Interestingly, **5**β only lost its anti-HSV-1 potential by 5- to 10-fold against a TK-deficient HSV-1 strain. An interesting antiviral activity was also noted for **5**β against vaccinia virus (EC_{50}: 2.2 μg/mL).

The compounds were also evaluated against a broad range of other viruses, including reovirus-1, Coxsackie virus B4, Sindbis virus, parainfluenza-3 virus, Punta Toro virus, vesicular stomatitis virus, and respiratory syncytial virus, but were found to be inactive at subtoxic concentrations.

Discussion

Compound **5**β displayed a C3′-endo/2′-exo twist (North type, $^3T_2$) sugar conformation, which has been shown to be a favorable conformation with respect to biological activity from studies employing the rigid methanocarbocyclic nucleosides.[19] As noted in Table 4, both **5**β and 4′S-T adopt the anti conformation. However, analysis of the torsion angle data for the χ(S−C1′−N1−C2) angle reveals a value of −76.0° for **5**β and −144.2 for 4′S-T. To investigate the origins of this conformational difference the Hydrogen-bonded assemblies for both structures were determined using PLATON software.[20]

Inspection of Figure 4 and correlation with the determined hydrogen bonded assemblies allows us to make the following statements:

- Both structures contain the common medium strength intermolecular interaction, H3···O3′.
- Both O5′ and O4 interact with hydroxyl (OH) hydrogens in both structures, but with different ones, to form medium strength intermolecular interactions.
- Both structures contain further weak intermolecular interactions of equal strength, H7C···O2 and H1′···O4 in **5**β, H2′B···O2 and H4′···O4 in 4′ S-T.
- Both structures contain the common intramolecular H1′···O2 interaction. Again, this bond is roughly of equal strength.
- The presence of the hydrogen-bond acceptor N6 in **5**β is contrasted to the presence of a C-H6 hydrogen-bond donor in 4′ S-T.

Table 5. Activity of Compounds against Varicella-zoster Virus (VZV) and Cytomegalovirus (CMV) in Human Embryonic Lung (HEL) Cell Cultures

	antiviral activity EC_{50}[a] (μM)							
	TK^+ VZV		TK^- VZV		CMV			
compound	YS strain	OKA strain	07/1 strain	YS/R strain	AD169 strain	Davis strain	MCC[b] μM	(CC_{50})[c] μM
5β	4.1	2.5	14	≥5[d]	>200	>200	>200	166
6β	>40	>40	>40	>40	>40	>40	>40	>40
7β	>40	>40	>40	>40	>40	>40	>40	>40
8	>40	>40	>40	>40	>40	>40	>40	>40
9	>40	>40	>40	>40	>40	>40	>40	>40
27	>40	>40	>40	>40	>40	>40	>40	>40
acyclovir	1.3	0.9	24	27	>200	>400	–	–
brivudin	0.003	0.003	29	56	>150	>400	–	–
ganciclovir	-	–	–	–	0.5	1.5	>150	>150

[a] Effective concentration required to reduce virus plaque formation by 50%. Virus input was 20 plaque forming units (PFU). [b] Minimum cytotoxic concentration that causes microscopically detectable alteration of cell morphology. [c] Cytotoxic concentration required to reduce cell growth by 50%. [d] No complete inhibition at higher drug concentrations.

Table 6. Activity of Compounds against Herpes Simplex Virus and Vaccinia Virus in E6SM Cell Cultures

	antiviral activity EC_{50} (μg/mL)[a]				
compound	HSV-1 (KOS)	HSV-2 (G)	HSV-1 TK^- (KOS ACV^r)	Vaccinia virus	MCC[b] (μg/mL)
5β	1.3	3.2	18	2.2	>200
6β	120	>200	>200	>200	>200
7β	>200	>200	120	>200	>200
8	120	120	120	120	>200
9	24	24	120	24	>200
27	>8	>8	>8	>8	≥ 40
brivudin	0.015	80	16	0.384	>400
acyclovir	0.076	0.076	48	>400	>400
ganciclovir	0.004	0.003	2.4	>100	>100

[a] Effective concentration required to reduce virus plaque formation by 50%. Virus input was 20 plaque forming units (PFU). [b] Minimum cytotoxic concentration that caused a microscopically detectable alteration of cell morphology.

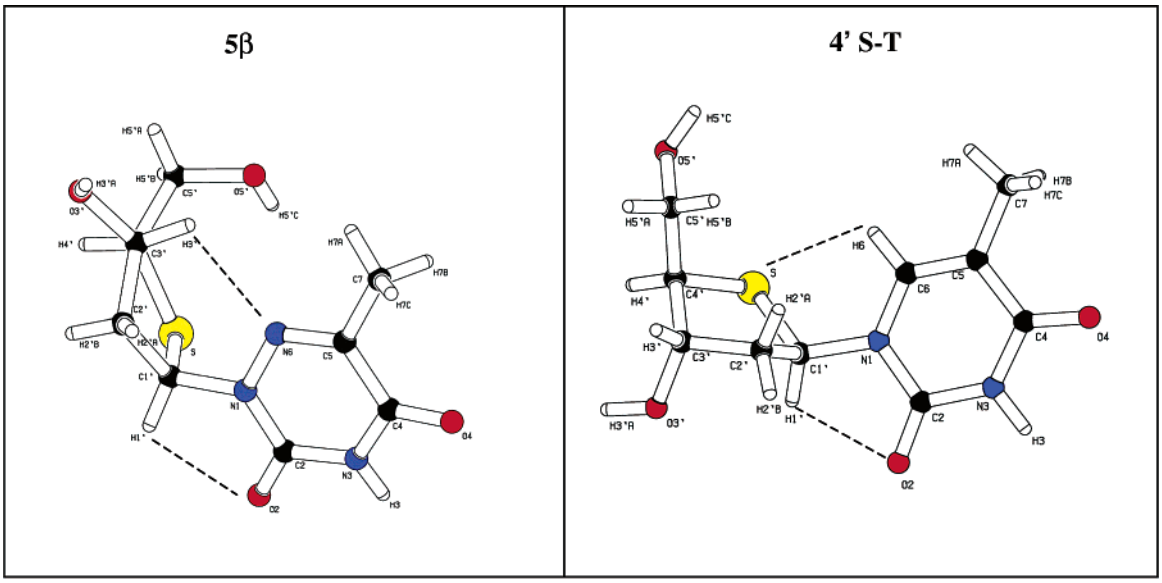


Figure 4. Both analogues contain the intramolecular bond, C1′-H1′···O2. However, the "scorpion-like" conformation of **5**β may be attributed to the intramolecular C3′−H3′···N6 bond.

In summary, therefore, it is proposed that the forces acting upon **5**β and 4′ S-T appear to be equal in terms of medium strength and weak intermolecular considerations. These structures even have one intramolecular interaction in common. The difference, however, lies in the presence of an intramolecular C−H3′···N6 bond in **5**β that is absent from 4′ S-T. It is this bond, therefore, that is thought to be responsible for the "scorpion-like" conformation noted in **5**β and shown in Figure 4.

This situation is analogous to that found in nucleotides by Rubin et al.[21] to explain the conformational preferences in nucleotide bases. In this case it was suggested that specific C−H···O interactions between the purine C8-H or pyrimidine C6-H and the oxygen O5′ are responsible for the anti conformation of these bases. Further evidence for the structural significance of this bond lies in the presence of the gauche conformation in the nucleoside ring of **5**β. Such conformational preferences are thought to be responsible for the packing differences found between **5**β and 4′ S-T.

Biologically **5**β and 4′ S-T both display antiviral activity vs herpes simplex virus; however, **5**β has a

much improved toxicity profile compared with 4′S-T. 4′S-T displayed significant cytotoxicity to rapidly proliferating cell populations (L1210, CCRF−CEM and human epidermoid carcinoma #2).[18] In contrast **5**β displayed cytotoxicity data comparable with ganciclovir (**5**β: MCC > 200 μM, CC_{50} 166 μM; ganciclovir: MCC and CC_{50} > 150 μM) in human embryonic lung (Table 5), and cytotoxicity comparable with acyclovir and brivudin (**5**β: MCC > 200 μg/mL; acyclovir/brivudin: MCC > 400 μg/mL) in E6SM cell cultures (Table 6).

Compound **5**β showed an interesting activity against herpes simplex virus type 1 and type 2 and varicella-zoster virus. Interestingly, it only lost antiviral efficacy by 5- to 15-fold against thymidine kinase-deficient HSV-1 and VZV strains. These observations may point to a partial dependence on virus-encoded TK for its eventual antiviral activity. These findings may also indicate that this compound may be inhibitory in its parental nucleoside form or use an alternative metabolic pathway for conversion to its antivirally active derivative. When evaluated for its inhibitory activity against phosphorylation of radiolabeled thymidine by purified cytosolic TK-1, mitochondrial TK-2, HSV-1 TK, and VZV TK, **5**β was not inhibitory toward TK-1, showed a marginal inhibitory effect toward TK-2 (IC_{50}: $\sim\geq$500 μM), but was endowed with a pronounced inhibitory potential toward HSV-1 TK (IC_{50}: 5.0 $\pm$ 0.94 μM) and VZV TK (IC_{50}: 33 $\pm$ 5.4 μM). These data suggest that the compound is most likely converted to its 5′-monophosphate by HSV-1 TK and VZV TK, but not cytosolic TK-1. Further studies are required to elucidate the metabolic characteristics of **5**β and to pinpoint its molecular target(s) against HSV and VZV.

Experimental Section

General Procedures. ^{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. FAB Mass spectra were determined by the EPSRC mass spectrometry center (Swansea, UK). Microanalyses were determined by Medac Ltd (Surrey, UK). Flash column chromatography was performed with silica gel 60 (230−400mesh) (Merck) and TLC was carried out on precoated silica plates (kiesel gel 60 F_{254}, BDH). Compounds were visualized by illumination under UV light (254 nm) or by the use of vanillin stain followed by charring on a hotplate. Melting points were determined on an electrothermal instrument and are uncorrected. All solvents were dried prior to use as described by the handbook Purification of Laboratory Chemicals[22] and stored over 4 Å molecular sieves, under nitrogen. Optical rotation was not undertaken for the final compounds **5**−**9** owing to the limited amounts of samples and the very limited additional information provided by such a measurement.

Semicarbazone of 2-Ketobutyric Acid (14). To a solution of semicarbazide·HCl **11** (10.92 g, 97.95 mmol) in water (60 mL) was added 2-ketobutyric acid **12** (10 g, 97.95 mmol) in small portions. The solution was stirred at room temperature for 1 h. The resulting white precipitate was filtered, washed with cold water, and dried under vacuum at 70 °C for 12 h to give 14.15 g (91%) of **14** as a white solid: mp: 180−182 °C (lit. mp: 188−189 °C); ^{1}H NMR (DMSO-d_6): δ 10.10 (s, 1, NH), 7.35 (bs, 1, NH_2), 6.70 (bs, 1, NH_2), 2.51 (q, 2, J = 7.5 Hz, CH_2) 0.90 (t, 3, J = 7.5 Hz, CH_3). ^{13}C NMR (DMSO-d_6): δ 164.95 (C=O, acid), 156.79 (C=O, amide), 140.86 (Cq), 18.02 (CH_2), 10.25 (CH_3).

Semicarbazone of 2-Ketovaleric Acid (15). To a solution of semicarbazide·HCl **11** (8.10 g, 72.63 mmol) in water (100 mL) was added 2-oxopentanoic acid sodium salt **13** (10 g, 72.41 mmol) in small portions. The suspension was heated to 100 °C, and ethanol (40 mL) was added in order to form a solution. The reaction was cooled to room temperature and then left in a refrigerator (4 °C) for 12 h. The resulting solid was filtered, washed with cold water, and dried under vacuum at 70 °C for 12 h to give 11.42 g (91%) of **15** as a white crystalline solid: mp: 200−202 °C (lit. mp: 175−176 °C); ^{1}H NMR (DMSO-d_6): δ 11.85 (s, 1, OH), 10.17 (s, 1, NH), 7.38 (bs, 1, NH_2), 6.68 (bs, 1, NH_2), 2.50 (t, 2, J = 7.6 Hz, CH_2), 1.35 (m, 2, J = 7.6 Hz, J = 7.3 Hz, CH_2), 0.87 (t, 3, J = 7.3 Hz, CH_3). ^{13}C NMR (DMSO-d_6): δ 165.10 (C=O, acid), 156.76 (C=O, amide), 139.59 (Cq), 26.32 (CH_2), 19.09 (CH_2), 13.98 (CH_3).

5-Ethyl-6-azauracil (16). To a solution of freshly prepared sodium ethoxide [prepared from the reaction of sodium (3.00 g, 130.4 mmol) and ethanol (90 mL)] was added a suspension of **14** (6.86 g, 43.38 mmol) in ethylene glycol (100 mL). The reaction was refluxed at 100 °C for 48 h and then concentrated in vacuo (high vacuum pump, 2.0 mmHg). The residue was dissolved in the minimal amount of hot water and acidified to pH 2 by the addition of concentrated hydrochloric acid. The solution was left in a refrigerator (4 °C) for 12 h, and the resulting white precipitate was filtered and dried in a vacuum oven at 70 °C for 12 h to give 2.3 g (38%) of **16** as a white crystalline solid: mp: 145−146 °C (lit. mp: 145−147 °C); ^{1}H NMR (DMSO-d_6): δ 12.03 (s, 1, NH), 11.56 (s, 1, NH), 2.46 (q, 2, J = 7.4 Hz, CH_2) 1.06 (t, 3, J = 7.4 Hz, CH_3). ^{13}C NMR (DMSO-d_6): δ 157.50 (C=O, C-4), 149.88 (Cq, C-5), 146.51 (C=O, C-5), 22.84 (CH_2), 10.62 (CH_3).

5-Propyl-6-azauracil (17). To a solution of freshly prepared sodium ethoxide [prepared from the reaction of sodium (4.6 g, 197.5 mmol) and ethanol (100 mL)] was added a suspension of **15** (11.4 g, 65.83 mmol) in ethylene glycol (200 mL). The reaction was refluxed at 100 °C for 72 h and then concentrated in vacuo (high vacuum pump, 2.0 mmHg). The residue was dissolved in the minimal amount of hot water and acidified to pH 2 by the addition of concentrated hydrochloric acid. The solution was left in a refrigerator for 12 h, and the resulting white precipitate was filtered and dried in a vacuum oven at 70 °C for 12 h to give 2.31 g (23%) (as determined from NMR of the mixture of product:starting material 5:1) of **17** as a white solid. NMR describes the product (**17**) signals only: ^{1}H NMR (DMSO-d_6): δ 12.04 (s, 1, NH), 11.87 (s, 1, NH), 2.41 (t, 2, J = 7.5 Hz, CH_2), 1.55 (m, 2, J = 7.5 Hz, J = 7.4 Hz, CH_2), 0.90 (t, 3, J = 7.4 Hz, CH_3). ^{13}C NMR (DMSO-d_6): δ 157.57 (C=O, C-4), 149.86 (Cq, C-5), 145.51 (C=O, C-2), 31.33 (CH_2), 19.48 (CH_2) 13.92 (CH_3).

General Procedure for the Synthesis of 1-(2-Deoxy-4-thio-3,5-di-*O*-benzyl-α/*β*-D-*erythro*-pentofuranosyl)-(6-aza-5-alkyl-uracil) (19, 20, and 21). To a suspension of 6-aza-5-alkyl-uracil (**10**, **16**, or **17**) (30 mmol) in anhydrous acetonitrile (30 mL) was added *N*,*O*-bis(trimethylsilyl)acetamide (60 mmol), and the reaction was stirred under nitrogen at room temperature for 1 h. To the now clear solution was added crushed 4 Å activated molecular sieves (2.5 g) followed by a solution of **18** (36 mmol) in anhydrous acetonitrile (60 mL). After 10 min, a solution of either iodine monochloride (36 mmol) or *N*-iodosuccinimide (36 mmol) in anhydrous acetonitrile (35 mL) (heat required to dissolve the *N*-iodosuccinimide) was added, and the resulting brown solution was stirred under nitrogen at room temperature for 24 h. The molecular sieves were removed by filtration, and the filtrate was concentrated in vacuo to give a brown syrup, which was dissolved in dichloromethane (300 mL) and washed with a saturated aqueous sodium thiosulfate solution (150 mL). The organic layer was dried ($MgSO_4$) and concentrated in vacuo to give a red/brown syrup. Purification by achieved by flash column chromatography.

1-(2-Deoxy-4-thio-3,5-di-*O*-benzyl-α/*β*-D-*erythro*-pentofuranosyl)-(6-aza-thymine) (19). Purification by flash column chromatography (petroleum ether−ethyl acetate 3:2 v/v) gave 13.54 g (78%) of the product **19** as a yellow hydroscopic solid, α and β mixture of approximately 1:1: R_f 0.18 (petroleum ether−ethyl acetate 3:2); ^{1}H NMR ($CDCl_3$) mixture of anomers: δ 10.17 (bs, 1, NH α and β), 7.38 (m, 10, CH Ar α and

β), 6.49 (dd, 1/2, $J_{1',2'} = 7.1$ Hz, $J_{1',2'} = 5.7$ Hz, H-1′ α/β), 6.34 (ψt, 1/2, $J_{1',2'} = 7.7$ and 7.5 Hz, H-1′ α/β), 4.62 (m, 4, CH_2 Bn α and β), 4.55 (m, 1/2, H-3′ β), 4.02 (m, 1, H-5′ α and β), 3.89 (dd, 1/2, $J = 9.7$ Hz, $J = 3.6$ Hz, H-3′ α), 3.79 (m, 1, H-5′ α and β), 3.62 (m, 1, H-4′ α and β), 2.65 (m, 1 1/2, H-2′ α and β), 2.46 (m, 1/2, H-2′ α/β), 2.32 (s, 1 1/2, CH_3 α/β), 2.24 (s, 1 1/2, CH_3 α/β). ^{13}C NMR ($CDCl_3$): δ 156.69, 156.65 (2 × C═O, C-4), 149.04, 148.92 (2 × Cq, C-5), 145.32, 145.00 (2 × C═O, C-2), 138.39, 138.36, 138.30, 138.17 (4 × Cq, Ar), 128.96, 128.90, 128.87, 128.42, 128.27, 128.23, 128.17, 128.10 (20 × CH, Ar), 82.98, 82.31 (2 × CH, C-3′), 73.70, 73.52, 73.14, 72.95 (8 × CH_2, Bn), 72.04, 70.96 (2 × CH_2, C-5′), 63.37, 59.94 (2 × CH, C-1′), 53.18, 52.92 (2 × CH, C-4′), 38.68, 37.73 (2 × CH_2, C-2′), 17.14, 17.02 (2 × CH_3). HRMS (ES+) (anomeric mixture) calcd for $C_{23}H_{26}N_3O_4S$ $(M + H)^+$ 440.1644, found, 440.1640.

1-(2-Deoxy-4-thio-3,5-di-*O*-benzyl-α/*β*-D-*erythro*-pentofuranosyl)-(5-ethyl-6-azauracil) (20). Purification by flash column chromatography (petroleum ether−ethyl acetate 7:3 v/v) gave 9.61 g (70%) of the product **20** as a thick syrup, α and β mixture of approximately 1:1: R_f 0.26 (petroleum ether−ethyl acetate 3:2); ^{1}H NMR ($CDCl_3$) mixture of anomers: δ 9.74 (s, 1, NH α and β), 7.37 (m, 10, CH Ar α and β), 6.50 (dd, 1/2, $J_{1',2'} = 7.3$ Hz, $J_{1',2'} = 5.4$ Hz, H-1′ α/β), 6.33 (t, 1/2, $J_{1',2'} = 7.6$ Hz, H-1′ α/β), 4.60 (m, 4 1/2, CH_2 Bn α and β, H-3′ β), 4.01 (m, 1, H-5′ α and β), 3.90 (m, 1/2, H-3′ α), 3.79 (m, 1, H-5′ α and β), 3.61 (m, 1, H-4′ α and β), 2.66 (m, 3 1/2, CH_2 α and β, H-2′ α/β), 2.48 (m, 1/2, H-2′ α/β), 1.29 (t, 1 1/2, $J = 7.4$ Hz CH_3 α/β), 1.17 (t, 1 1/2, $J = 7.4$ Hz CH_3 α/β). ^{13}C NMR ($CDCl_3$): δ 156.27 (2 × C═O, C-4), 148.87, 148.76, 148.65, 145.61 (2 × C═O, C-2) and (2 × Cq, C-5), 138.35, 138.29, 138.17, (4 × Cq, Ar), 128.93, 128.90, 128.85, 128.41, 128.25, 128.22, 128.16, 128.07 (20 × CH, Ar), 83.10, 82.32 (2 × CH, C-3′), 73.68, 73.56, 73.18, 73.01 (4 × CH_2, Bn), 72.11, 7088 (2 × CH_2, C-5′), 63.25, 59.98 (2 × CH, C-1′), 53.16, 52.87 (2 × CH, C-4′), 38.63, 37.91 (2 × CH_2, C-2′), 23.99, 23.85 (2 × CH_2), 10.73, 10.57 (2 × CH_3). Anal. ($C_{24}H_{27}N_3O_4S$) C, H, N, S.

1-(2-Deoxy-4-thio-3,5-di-*O*-benzyl-α/*β*-D-*erythro*-pentofuranosyl)-(5-propyl-6-azauracil) (21). Purification by flash column chromatography (petroleum ether−ethyl acetate 7:3 v/v) gave 4.37 g (76%) of the product **21** as a thick orange syrup, α and β mixture of approximately 1:1: R_f 0.30 (petroleum ether−ethyl acetate 3:2); ^{1}H NMR ($CDCl_3$) mixture of anomers: δ 9.69 (s, 1, NH α and β), 7.34 (m, 10, CH Ar α and β), 6.48 (dd, 1/2, $J_{1',2'} = 7.1$ Hz, $J_{1',2'} = 5.5$ Hz, H-1′ α/β), 6.30 (t, 1/2, $J = 7.6$ Hz, H-1′ α/β), 4.58 (m, 4, CH_2 Bn α and β), 4.53 (m, 1/2, H-3′ α/β), 3.98 (m, 1, H-5′ α and β), 3.88 (m, 1/2, H-3′ α/β), 3.77 (m, 1, H-5′ α and β), 3.60 (m, 1, H-4′ α and β), 2.64 (m, 3 1/2, CH_2 α and β, H-2′ α/β), 2.46 (m, 1/2, H-2′ α/β), 1.68 (m, 2, CH_2 α and β), 1.00 (m, 3, CH_3 α and β). ^{13}C NMR ($CDCl_3$): δ 156.37, 156.33 (2 × C═O, C-4), 148.80, 148.69 (2 × Cq, C-5), 148.00, 147.66 (2 × C═O, C-4), 138.36, 138.31, 138.19, (4 × Cq, Ar), 128.94, 128.89, 128.85, 128.41, 128.25, 128.22, 128.15, 128.05 (20 × CH, Ar), 83.04, 82.32 (2 × CH, C-3′), 73.66, 73.55, 73.20, 73.05 (4 × CH_2, Bn), 72.06, 70.86 (2 × CH_2, C-5′), 63.22, 59.89 (2 × CH, C-1′), 53.16, 52.84 (2 × CH, C-4′), 38.65, 37.91 (2 × CH_2, C-2′), 32.26, 32.14 (2 × CH_2), 19.90, 19.67 (2 × CH_2), 14.15, 14.13 (2 × CH_3). Anal. ($C_{25}H_{29}N_3O_4S$) C, H, N, S.

General Procedure for the Synthesis of 1-(2-Deoxy-4-thio-α- and *β*-D-*erythro*-pentofuranosyl)-(6-aza-5-alkyluracil) (5, 6, and 7). To a cooled (−80 °C) solution of boron trichloride (1 M in dichloromethane, 25 mL, 25 mmol) in anhydrous dichloromethane (25 mL) was added dropwise a solution of **19**, **20**, or **21** (10 mmol) in anhydrous dichloromethane (25 mL). The reaction was stirred at −80 °C for 4 h, and then the reaction was quenched by adding a 1:1 mixture of methanol/dichloromethane (50 mL) dropwise at −80 °C and allowed to warm to room temperature. The reaction was concentrated in vacuo to give a brown solution, which was coevaporated with methanol (3 × 15 mL) to give a brown syrup. Purification was achieved by flash column chromatography.

1-(2-Deoxy-4-thio-α- and *β*-D-*erythro*-pentofuranosyl)-(6-azathymine) (5). Purification by flash column chromatography (chloroform−methanol 9: 1 v/v) gave the product **5** as the anomers α (pale yellow solid) and β (off white solid). Yield: **5**α 3.45 g (43%) and **5***β* 1.56 g (20%); mp **5**α 153−155 °C and **5***β* 153 °C (decomposes); R_f **5**α 0.22 and **5***β* 0.18 (chloroform/methanol 9:1); α-*anomer* **5**α : ^{1}H NMR (CD_3OD): δ 6.34 (dd, 1, $J_{1',2'} = 8.1$ Hz, $J_{1',2'} = 5.9$ Hz, H-1′), 4.22 (m, 1, H-3′), 3.80 (dd, 1, $J_{5',5'} = 10.1$ Hz, $J_{5',4'} = 4.3$ Hz, H-5′), 3.60 (m, 2, H-5′ and H-4′), 2.65 (m, 1, H-2′), 2.51 (m, 1, H-2′), 2.24 (s, 3, CH_3). ^{13}C NMR (CD_3OD): δ 158.63 (C═O, C-4), 150.62 (Cq, C-5), 146.67 (C═O, C-2), 76.73 (CH, C-3′), 65.27 (CH_2, C-5′), 62.16 (CH, C-1′), 59.83 (CH, C-4′), 41.46 (CH_2, C-2′), 16.98 (CH_3). UV (MeOH) $\lambda_{max} = 296$ nm (ϵ 1770), 290 nm (ϵ 1530). HRMS (CI) Calcd for $C_9H_{17}N_4O_4S$ $(M + H)^+$ 260.0705, found 260.0710. *β-anomer* **5***β*: ^{1}H NMR (CD_3OD): δ 6.37 (dd, 1, $J_{1',2'} = 7.3$ Hz, $J_{1',2'} = 5.1$ Hz, H-1′), 4.71 (m, 1, H-3′), 3.89 (dd, 1, $J_{5',5'} = 11.3$ Hz, $J_{5',4'} = 6.8$ Hz, H-5′), 3.68 (dd, 1, $J_{5,5} = 11.2$ Hz, $J_{5,4} = 6.9$ Hz, H-5′) 3.39 (m, 1, H-4′), 2.70 (m, 1, H-2′), 2.35 (m, 1, H-2′), 2.64 (s, 3, CH_3). ^{13}C NMR (CD_3OD): δ 158.70 (C═O, C-4), 150.77 (Cq, C-5), 146.04 (C═O, C-2), 76.74 (CH, C-3′), 66.22 (CH_2, C-5′), 63.60 (CH, C-1′), 59.53 (CH, C-4′), 41.72 (CH_2, C-2′), 17.00 (CH_3). UV (MeOH) $\lambda_{max} = 296$ nm (ϵ 1370), 291 nm (ϵ 1310). Anal. ($C_9H_{13}N_3O_4S$) C, H, N, S.

1-(2-Deoxy-4-thio-α- and *β*-D-*erythro*-pentofuranosyl)-(5-ethyl-6-azauracil) (6). Purification by flash column chromatography (chloroform−methanol 95:5 v/v) gave the product **6** as the anomers, α (white solid) and β (white solid). Yield: **6**α 2.31 g (40%) and **6***β* 1.01 g (17%); mp **6**α 68−70 °C and **6***β* 189−191 °C; R_f **6**α 0.42 and **5***β* 0.35 (chloroform/methanol 4:1); α-*anomer* **6**α : ^{1}H NMR (DMSO-d_6): δ 12.15 (s, 1, NH), 6.13 (t, 1, $J = 7.7$ Hz, H-1′), 5.30 (d, 1, $J = 6.6$ Hz, OH), 4.87 (ψt, 1, $J = 5.2$ Hz and $J = 4.9$ Hz, OH), 3.92 (m, 1, H-3′), 3.82 (m, 1, H-5′), 3.45 (m, 1, H-5′), 3.33 (m, 1, H-4′), 2.49 (m, 4, CH_2 and H-2′), 1.14 (t, 3, $J = 7.4$ Hz, CH_3). ^{13}C NMR (DMSO-d_6): δ 156.60 (C═O, C-4), 148.69 (Cq, C-5), 147.71 (C═O, C-2), 72.25 (CH, C-3′), 63.52 (CH_2, C-5′), 58.84 (CH, C-1′), 57.88 (CH, C-4′), 39.81 (CH_2, C-2′), 23.24 (CH_2), 10.40 (CH_3). UV (MeOH) $\lambda_{max} = 295$ nm (ϵ 1820), 291 nm (ϵ 1850). HRMS (CI) Calcd for $C_{10}H_{15}N_3O_4S$ $(M + H)^+$ 274.0861, found 274.0863. *β-anomer* **6***β*: ^{1}H NMR (DMSO-d_6): δ 12.13 (s, 1, NH), 6.19 (ψt, 1, $J = 6.8$ Hz and $J = 5.5$ Hz, H-1′), 5.21 (d, 1, $J = 4.0$ Hz, OH), 4.96 (bs, 1, OH), 4.49 (m, 1, H-3′), 3.70 (ψt, 1, $J = 8.4$ Hz and $J = 7.7$ Hz, H-5′), 3.38 (m, 1, H-5′), 3.21 (m, 1, H-4′), 2.50 (m, 3, CH_2 and H-2′), 2.17 (m, 1, H-2′), 1.12 (t, 3, $J = 7.3$ Hz, CH_3). ^{13}C NMR (DMSO-d_6): δ 156.62 (C═O, C-4), 148.72 (Cq, C-5), 147.29 (C═O, C-2), 74.44 (CH, C-3′), 64.92 (CH_2, C-5′), 61.58 (CH, C-1′), 58.69 (CH, C-4′), 40.29 (CH_2, C-2′), 23.11 (CH_2), 10.21 (CH_3). UV (MeOH) $\lambda_{max} = 295$ nm (ϵ 1710), 291 nm (ϵ 1710). HRMS (CI) Calcd for $C_{10}H_{15}N_3O_4S$ $(M + H)^+$ 274.0861, found 274.0865.

1-(2-Deoxy-4-thio-α- and *β*-D-*erythro*-pentofuranosyl)-(5-propyl-6-azauracil) (7). Purification by flash column chromatography (chloroform−methanol 96:4 v/v) gave the product **7** as the anomers, α (white solid) and β (white solid). Yield: **7**α 1.07 g (40%) and **7***β* 0.84 g (31%); mp **7**α 125−126 °C and **7***β* 134−135 °C; R_f **7**α 0.47 and **7***β* 0.42 (chloroform/methanol 4:1); α-*anomer* **7**α: ^{1}H NMR (CD_3OD): δ 6.13 (dd, 1, $J_{1',2'} = 8.2$ Hz, $J_{1',2'} = 5.9$ Hz, H-1′), 4.30 (m, 1, H-5′), 3.89 (m, 1, H-5′), 3.68 (m, 2, H-5′ and H-4′), 2.73 (m, 3, CH_2 and H-2′), 2.61 (m, 1, H-2′), 1.86 (m, 2, $J = 7.4$ Hz, CH_2), 1.11 (t, 3, $J = 7.4$ Hz, CH_3). ^{13}C NMR (CD_3OD): δ 158.39 (C═O, C-4), 150.49 (Cq, C-5), 149.27 (C═O, C-2), 76.73 (CH, C-3′), 65.24 (CH_2, C-5′), 62.10 (CH, C-1′), 59.81 (CH, C-4′), 41.46 (CH_2, C-2′), 33.22 (CH_2), 21.01 (CH_2), 14.43 (CH_3). UV (MeOH) λ_{max} = 297 nm (ϵ 2260), 291 nm (ϵ 2030). Anal. ($C_{11}H_{17}N_3O_4S$) C, H, N, S. *β-anomer* **7***β*: ^{1}H NMR (CD_3OD): δ 6.30 (dd, 1, $J_{1',2'} = 7.3$ Hz, $J_{1',2'} = 4.7$ Hz, H-1′), 4.64 (m, 1, H-3′), 3.82 (m, 1, $J_{5',5'} = 11.2$ Hz, H-5′), 3.59 (m, 1, $J_{5',5'} = 11.2$ Hz, H-5′), 3.34 (m, 1, H-4′), 2.57 (m, 3, CH_2 and H-2′), 2.29 (m, 1, H-2′), 1.69 (m, 2, CH_2), 0.99 (t, 3, $J = 7.3$ Hz, CH_3). ^{13}C NMR (CD_3OD): δ 158.48 (C═O, C-4), 150.61 (Cq, C-5), 148.57 (C═O, C-2), 76.76 (CH, C-3′), 66.33 (CH_2, C-5′), 63.40 (CH, C-1′), 59.44 (CH, C-4′), 41.71 (CH_2, C-2′), 33.11 (CH_2), 20.82 (CH_2), 14.47 (CH_3). UV (MeOH) $\lambda_{max} = 296$ nm (ϵ 2180), 291 nm (ϵ 1980). Anal. ($C_{11}H_{17}N_3O_4S$) C, H, N, S.

General Procedure for the Synthesis of 1-(2-Deoxy-2-iodo-4-thio-α/β-D-*erythro*-pentofuranosyl)-(6-aza-5-alkyluracil) (23, 24, and 25). To a 0 °C solution of **18** (10 mmol) in anhydrous dichloromethane (55 mL) was added 2,6-di-*tert*-butyl-4-methylpyridine (15 mmol) followed by the dropwise addition of iodine monochloride (12 mmol) in anhydrous dichloromethane (13 mL) over 10 min under nitrogen. The reaction was then stirred at 0 °C for 5 h. A freshly prepared solution of bisilylated 6-azapyrimidine [prepared by the reaction of 6-azapyrimidine **10**, **16**, or **17** (15 mmol) in dichloromethane (30 mL) and *N*,*O*-bis(trimethylsilyl)acetamide (30 mmol)] was added dropwise followed by a further addition of iodine monochloride (12 mmol). The reaction was warmed to room temperature and stirred under nitrogen for 16 h. The reaction was quenched by the addition of saturated aqueous sodium thiosulfate (50 mL) and the product extracted by washing with dichloromethane (3 × 25 mL). The organic layer was dried ($MgSO_4$) and concentrated in vacuo to give an orange syrup. Purification was achieved by flash column chromatography.

1-(2-Deoxy-2-iodo-4-thio-3, 5-di-*O*-benzyl-α/β-D-*erythro*-pentofuranosyl)-(6-azathymine) (23). Purification by flash column chromatography (petroleum ether−ethyl acetate 7:3 v/v) gave 3.48 g (70%) of **23** as a thick orange syrup, α and β mixture of approximately 1:4: R_f 0.32 (petroleum ether−ethyl acetate 3:2); *Anomeric mixture, α:β, 1:4, Major anomer (β) signals described* ^{1}H NMR ($CDCl_3$) δ 9.92 (s, 4/5, NH β), 7.52 (m, 10, CH Ar β), 6.61 (d, 4/5, $J_{1′,2′}$ = 8.4 Hz, H-1′ β), 5.01 (dd, 4/5, $J_{2′,1′}$ = 8.4 Hz, $J_{2′,3′}$ = 3.9 Hz, H-2′ β), 4.83 (s, 8/5, CH_2Ph β), 4.69 (s, 8/5, CH_2Ph β), 4.25 (ψt, 4/5, J = 3.5 and 2.8 Hz, H-3′ β), 3.94 (m, 4/5, $J_{4′,5′}$ = 7.0 Hz, $J_{4′,3′}$ = 2.5 Hz, H-4′ β), 3.75 (d, 8/5, $J_{5′,5′}$ = 7.0 Hz, $J_{5′,4′}$ = 2.5 Hz, H-5′ β), 2.36 (s, 12/5, CH_3 β). *Major anomer (β) signals described* ^{13}C NMR ($CDCl_3$) δ 156.31 (C═O, C-4), 148.81 (Cq, C-5), 145.66 (C═O, C-2), 138.12, 137.48 (2 × Cq, Ar), 128.94, 128.85, 128.73, 128.48, 128.35, 128.08 (10 × CH, Ar), 82.95 (CH, C-3′), 73.65, 72.73, (2 × CH_2, Bn), 72.44 (CH_2, C-5′), 69.35 (CH, C-1′), 50.48 CH, C-4′), 29.44 (CH_2, C-2′), 17.12 (CH_3). HRMS (ES+) Calcd for $(M + H)^+$ 566.0611, found 566.0594.

1-(2-Deoxy-2-iodo-4-thio-3,5-di-*O*-benzyl-α/β-D-*erythro*-pentofuranosyl)-(5-ethyl-6-azauracil) (24). Purification by flash column chromatography (petroleum ether−ethyl acetate 7:3 v/v) gave 2.9 g (67%) of **24** as a thick orange syrup, α and β mixture of approximately 1:3: R_f 0.42 (petroleum ether−ethyl acetate 3:2); *Anomeric mixture, α:β, 1:4, Major anomer (β) signals described* ^{1}H NMR ($CDCl_3$) δ 9.65 (bs, 1, NH β), 7.33 (m, 10, CH Ar β), 6.47 (d, 3/4, $J_{1′,2′}$ = 8.2 Hz, H-1′ β), 4.86 (dd, $J_{2′,1′}$ = 8.2 Hz, $J_{2′,3′}$ = 3.9 Hz, H-2′ β), 4.67 (s, 11/2, CH_2Ph β), 4.10 (t, 3/4, $J_{3′,2′}$ = $J_{3′,4′}$ = 3.3 Hz, H-3′), 3.79 (m, 1, $J_{4′,5′}$ = 6.9 Hz, $J_{4′,3′}$ = 2.6 Hz, H-4′β), 3.58 (d, 11/2, $J_{5′,4′}$ = 7.0 Hz, H-5′ β), 2.63 (m, 11/2, CH_2β), 1.13 (t, 21/4, J = 7.3 Hz, CH_3 β). ^{13}C NMR ($CDCl_3$) δ 156.05 (2 × C═O, C-4), 149.95, 149.18 (2 × Cq, C-5), 148.97, 148.70 (2 × C═O, C-2), 138.07, 137.95, 137.49 (4 × Cq, Ar), 128.94, 128.90, 128.85, 128.66, 128.46, 128.36, 128.09 (20 × CH, Ar), 87.33, 83.07 (2 × CH, C-3′), 75.20, 73.84, 73.67, 72.73, (4 × CH_2, Bn), 72.47, 70.49 (CH_2, C-5′), 69.52, 64.34 (2 × CH, C-1′), 50.46, 50.22 (2 × CH, C-4′), 29.27, 29.17 (2 × CH_2, C-2′), 24.04, 23.92 (2 × CH_2) 10.72, 10.48 (2 × CH_3). HRMS (ES+) Calcd for $(M + H)^+$ 580.0767, found: 580.0767.

1-(2-Deoxy-2-iodo-4-thio-3,5-di-*O*-benzyl-α/β-D-*erythro*-pentofuranosyl)-(5-propyl-6-azauracil) (25). Purification by flash column chromatography (petroleum ether−ethyl acetate 7:3 v/v) gave 3.37 g (67%) of **25** as a thick orange syrup, α and β mixture of approximately 1:3. R_f 0.43 (petroleum ether−ethyl acetate 7:3); *Anomeric mixture, α:β, 1:3, Major anomer (β) signals described* ^{1}H NMR ($CDCl_3$) δ 9.48 (s, 3/4, NH β), 7.43 (m, 10, CH_2Phβ), 6.56 (d, 3/4, $J_{1′,2′}$ = 8.5 Hz, H-1′ β), 4.93 (dd, 3/4, $J_{2′,1′}$ = 8.5 Hz, $J_{2′,3′}$ = 3.9 Hz, H-2′β), 4.76 (s, 11/2, CH_2Ph β), 4.61 (s, 11/2, CH_2Ph β), 4.19 (ψt, 3/4, $J_{3′,2′}$ = 3.5 Hz, $J_{3′,4′}$ = 2.7 Hz, H-3′ β), 3.88 (dd, 1, $J_{4′,5′}$ = 7.1 Hz, $J_{4′,3′}$ = 2.4, H-4′ β), 3.66 (d, 11/2, $J_{5′,4′}$ = 7.1 Hz, H-5′ β), 2.68 (m, 11/2, CH_2 β), 1.71 (q, 11/2, CH_2 β), 1.04 (t, 2 1/4, CH_3 β). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.28, 156.01 (2 × C═O, C-4), 148.89, 148.59, 148.20 (2 × Cq, C-5 and 2 × C═O, C-2), 138.68, 138.07, 137.86, 137.49 (4 × Cq, Ar), 128.94, 128.91, 128.84, 128.67, 128.45, 128.36, 128.07 (20 × CH, Ar), 87.26, 83.04 (2 × CH, C-3′), 75.19, 73.84, 73.66, 72.70, (4 × CH_2, Bn), 72.55, 70.48 (2 × CH_2, C-5′), 69.37, 64.22 (2 × CH, C-1′), 50.41, 50.21 (2 × CH, C-4′), 32.24, 32.12 (2 × CH_2), 29.17, 29.10 (2 × CH, C-2′), 19.95, 19.61 (2 × CH_2), 14.09 (2 × CH_3). HRMS (ES+) Calcd for $(M + NH_4)^+$ 611.1189, found: 611.1187.

1-(2-Deoxy-3,5-di-*O*-acetyl-4-thio-β-D-*erythro*-pentofuranosyl)-(6-azathymine) (26). To a solution of **5**β (1.5 g, 5.79 mmol) in anhydrous pyridine (20 mL) was added acetic anhydride (2.1 mL, 23.14 mmol). The reaction was stirred at 0 °C for 15 min and then at room temperature for 16 h. The reaction was quenched with *N*,*N*-dimethylethylenediamine (4 mL, 37.58 mmol) in dichloromethane (50 mL) while cooling the reaction flask in ice. The organic layer was then washed with 2 M aqueous HCl (30 mL), saturated aqueous sodium bicarbonate (30 mL), and water (30 mL), dried ($MgSO_4$), and concentrated in vacuo to give a yellow liquid, which was azeotroped with toluene to give a yellow syrup. Purification by flash column chromatography (chloroform−methanol 19:1 v/v) gave 1.50 g (75%) of **26** as a pale yellow syrup: R_f 0.69 (chloroform/methanol 4:1); ^{1}H NMR ($CDCl_3$): δ 10.23 (bs, 1, NH), 6.54 (ψt, 1, J = 7.0 Hz, J = 6.4 Hz, H-1′), 5.63 (m, 1, H-3′), 4.37 (dd, 1, $J_{5′,5′}$ = 11.4 Hz, $J_{5′,4′}$ = 8.6 Hz, H-5′), 4.26 (dd, 1, $J_{5′,5′}$ = 11.5 Hz, $J_{5′,4′}$ = 6.6 Hz, H-5′), 3.70 (m, 1, H-4′), 2.82 (m, 1, H-2′), 2.48 (m, 1, H-2′), 2.20 (s, 3, CH_3), 2.14 (s, 3, CH_3 Ac), 2.12 (s, 3, CH_3 Ac). ^{13}C NMR ($CDCl_3$): δ 171.15 (C═O, Ac), 170.85 (C═O, Ac), 156.54 (C═O, C-4), 149.07 (C═O, C-2), 145.82 (Cq, C-5), 77.95 (CH, C-3′), 65.87 (CH_2, C-5′), 63.33 (CH, C-1′), 52.15 (CH, C-4′), 38.34 (CH_2, C-2′), 21.55 (CH_3, Ac), 21.22 (CH_3, Ac), 17.14 (CH_3). HRMS (ES+) Calcd for $(M + NH_4)^+$ 361.1182, found 361.1181.

1-(2-Deoxy-3,5-di-*O*-acetyl-4-thio-β-D-*erythro*-pentofuranosyl)-(4-thio-6-azathymine) (27). To a solution of **26** (1.65 g, 4.81 mmol) in anhydrous toluene (20 mL) was added Lawesson's Reagent (2.33 g, 5.77 mmol), and the reaction was refluxed under nitrogen for 3 h. The reaction was quenched by coevaporation with methanol to give a red/brown syrup. Purification by flash column chromatography (dichloromethane−ethyl acetate 94:6 v/v) gave 1.10 g (64%) of **27** as a bright orange hydroscopic solid. A sample was prepared for high-resolution mass spectrometry and testing by preparative TLC. R_f 0.33 (dichloromethane/ethyl acetate 9:1); ^{1}H NMR ($CDCl_3$): δ 10.90 (bs, 1, NH), 6.57 (ψt, 1, J = 6.9 Hz, J = 6.4 Hz, H-1′), 5.71 (m, 1, H-3′), 4.45 (dd, 1, $J_{5′,5′}$ = 11.3 Hz, $J_{5′,4′}$ = 8.6 Hz, H-5′), 4.33 (dd, 1, $J_{5′,5′}$ = 11.5 Hz, $J_{5′,4′}$ = 6.6 Hz, H-5′), 3.76 (m, 1, H-4′), 2.91 (m, 1, H-2′), 2.55 (m, 1, H-2′), 2.53 (s, 3, CH_3), 2.20 (s, 3, CH_3 Ac), 2.19 (s, 3, CH_3 Ac). ^{13}C NMR ($CDCl_3$): δ 182.67 (C═S, C-4), 171.18 (C═O, Ac) 170.80 (C═O, Ac), 148.78 (Cq, C-5), 146.05 (C═O, C-2), 78.01 (CH, C-3′), 65.85 (CH_2, C-5′), 63.67 (CH, C-1′), 52.24 (CH, C-4′), 38.44 (CH_2, C-2′), 21.57 (CH_3, Ac), 21.25 (CH_3, Ac), 20.83 (CH_3). HRMS (ES+) Calcd for $(M + NH_4)^+$ 377.0953, found 377.0949.

1-(2-Deoxy-4-thio-β-D-*erythro*-pentofuranosyl)-(4-thio-6-azathymine) (8). Compound **27** (1 g, 2.78 mmol) was dissolved in methanolic ammonia (50 mL) at 0 °C and stirred under nitrogen at 0 °C for 4 h. The reaction was warmed to room temperature where it was stirred under nitrogen for a further 4 h. The reaction was concentrated in vacuo to give an orange foam. Purification by flash column chromatography (dichloromethane−ethyl acetate 94:6 v/v) gave 0.5 g (66%) of the product as a pale orange hydroscopic solid: R_f 0.57 (chloroform/methanol 3:2); ^{1}H NMR (CD_3OD): δ 6.29 (dd, 1, $J_{1′,2′}$ = 7.3 Hz, $J_{1′,2′}$ = 5.0 Hz, H-1′), 4.68 (m, 1, H-3′) 3.86 (dd, 1, $J_{5′,5′}$ = 11.3 Hz, $J_{5′,4′}$ = 6.7 Hz, H-5′), 3.65 (dd, 1, $J_{5′,5′}$ = 11.3 Hz, $J_{5′,4′}$ = 6.9 Hz, H-5′), 3.39 (m, 1, H-4′), 2.69 (m,1, H-2′), 2.38 (s, 3, CH_3), 2.37 (m, 1, H-2′). ^{13}C NMR (CD_3OD): δ 185.39 (C═S, C-4), 148.95 (Cq, C-5), 147.750 (C═O, C-2), 76.78 (CH, C-3′), 66.17 (CH_2, C-5′), 63.85 (CH, C-1′), 59.60 (CH, C-4′), 41.77 (CH_2, C-2′), 20.92 (CH_3). UV (MeOH) λ_{max} = 297 nm (ϵ 1730), 291 nm (ϵ 1590). HRMS (ES+) Calcd for $(M + NH_4)^+$ 293.0742, found 293.0738.

1-(2-Deoxy-4-thio-β-D-*erythro*-pentofuranosyl)-(5-methyl-6-azacytosine) (9). Compound **27** (0.5 g, 1.82 mmol) was

dissolved in methanolic ammonia (30 mL) and heated at 30 °C in a sealed vessel for 72 h. The reaction was concentrated in vacuo to give a pale orange solid. Trituration with acetone and methanol gave 0.44 g (93%) of the product as a cream solid: mp 68 °C (decomp); R_f 0.39 (chloroform−methanol 3:2); ^{1}H NMR (DMSO-d_6): δ 8.04 (bs, 1, NH_2), 7.48 (bs, 1, NH_2), 6.21 (ψt, 1, J = 6.7 Hz, J = 6.0 Hz, H-1′), 5.16 (bs, 1, OH) 4.96 (bs, 1, OH), 4.45 (m, 1, H-3′) 3.66 (dd, 1, $J_{5',5'}$ = 10.9 Hz, $J_{5',4'}$ = 7.0 Hz, H-5′), 3.36 (dd, 1, $J_{5',5'}$ = 10.8 Hz, $J_{5',4'}$ = 7.3 Hz, H-5′), 3.20 (m, 1, $J_{4',5'}$ = 7.0 Hz, H-4′), 2.46 (m, 1, H-2′), 2.16 (s, 3, CH_3), 2.11 (m, 1, H-2′). ^{13}C NMR (DMSO-d_6): δ 158.64 (C=O), C-2), 154.06 (Cq, C-4), 134.28 (Cq, C-5), 74.40 (CH, C-3′), 64.98 (CH_2, C-5′), 62.06 (CH, C-1′), 58.42 (CH, C-4′), 40.37 (CH_2, C-2′), 17.89 (CH_3). UV (MeOH) λ_{max} = 296 nm (ϵ 2240), 291 nm (ϵ 2130). HRMS (ES+) Calcd for $(M + Na)^+$ 281.0684, found 281.0687.

X-ray Structure Determination. A single-crystal suitable for X-ray diffraction was grown by slow evaporation in a narrow-necked tube using a saturated solution of **5**β in deuterated methanol. The X-ray measurements were made on a crystal of approximate dimensions 0.20 × 0.20 × 0.10 mm. Intensity data were obtained using graphite monochromated Mo Kα radiation (λ = 0.71073 Å) at 150 K and a Bruker-Nonius *Kappa*CCD area detector diffractometer. ϕ and ω scans were employed to fill the Ewald sphere using COLLECT software.[23] Unit cell refinement and data reduction were undertaken using DENZO and SCALEPACK software.[24,25]

Compound **5**β ($C_9H_{13}N_3O_4S$), FW = 259.28, crystallized in the orthorhombic space group $P2_12_12_1$, with a = 7.7150(10), b = 8.3080(10), c = 17.9590(10) Å, V = 1151.1(2) Å^3, Z = 4, D_{calcd} = 1.496 mg/m^3 and μ (Mo Kα) = 0.289 mm^{-1}. The total data recorded within θ range of 3.48 to 27.49° was 6187, which merged to give 2572 unique data (R_{int} = 0.034) of which 2431 were considered observed ($F_o > 2\sigma(F)$). A semiempirical scan absorption correction was applied using SORTAV[26] and values of T_{min} = 0.9444 and T_{max} = 0.9717 were obtained. The structure was solved using SHELX-S[27] and refined using SHELXL[28] to give a final *R1* value of 0.0300 for 206 parameters. The absolute structure parameter[29] was 0.01(6). The non-hydrogen atoms were refined anisotropically. H atoms were found using the difference map and their positions refined isotropically.

Atomic coordinates, displacement factor coefficients, a full list of bond lengths, angles, and torsion angle values are included as Supporting Information. Also included is an ORTEP III for Windows[30] representation (50% probability) of **5**β.

Conformational Analysis. Phase angle and degree of amplitude (P and v_m) were determined using the eqs i and ii, respectively, as described by Altona and Sundaralingam.[16]

$$\tan P = (v_4 + v_1) - (v_3 + v_0)/2v_2(\sin 36 + \sin 72) \quad \text{(i)}$$

$$v_2 = v_m \cos P \quad \text{(ii)}$$

Thymidine Kinase Assay. The radiolabeled substrate [*methyl*-^{3}H]dThd (70 Ci/mmol) was obtained from Amersham Pharmacia Biotech. The thymidine kinase activity using purified cytosolic TK-1, recombinant mitochondrial TK-2, recombinant herpes simplex virus type 1 TK, and recombinant varicella-zoster virus TK was assayed in a 50 μL reaction mixture containing 50 mM Tris·HCl, pH 8.0, 2.5 mM $MgCl_2$, 10 mM DTT, 0.5 mM CHAPS, 3 mg/mL bovine serum albumin, 2.5 mM ATP, 1 μM [*methyl*-^{3}H]dThd, and varying concentrations of compound **5**β and enzyme. The samples were incubated at 37 °C for 30 min. Aliquots of 45 μL of the reaction mixtures were spotted on Whatman DE−81 filter paper disks. The filters were washed three times for 5 min in 1 mM CH_3COONH_4 and once for 5 min in ethanol. The radioactivity was determined by scintillation counting.

Cell Cultures. 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.[31−34] 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.

The 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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Supporting Information Available: X-ray crystallographic data for compound **5**β. Tables of calculated H-bonded assemblies for **5**β and 4′ S-T using PLATON software and a figure for comparison of H-bonded assemblies of **5**β and 4′ S-T. A table of elemental analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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