Synthesis and Antiviral Activity of the Carbocyclic Analogue of the Highly Potent and Selective Anti-VZV Bicyclo Furano Pyrimidines

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Carbocyclic nucleoside analogues are catabolically stable since they are resistant to phosphorolytic cleavage by pyrimidine nucleoside phosphorylase enzymes. The carbocyclic analogue (C-BCNA) of the highly potent and selective anti-VZV bicyclic nucleoside analogue (BCNA) 6-pentylphenylfuro[2,3-*d*]pyrimidine-2′-deoxyribose was synthesized using carbocyclic 2′-deoxyuridine as starting material. C-BCNA was found to be chemically more stable than the furano lead, but it was shown to be significantly less antivirally active than its parent nucleoside analogue. It was noted to have a 10-fold lower inhibitory activity against the VZV-encoded thymidine kinase. This reduction of activity may be attributed to the different conformation of the sugar and base, as predicted by computational studies and supported by NMR studies. However, other factors besides affinity for VZV-TK must account for the greatly reduced antiviral potency.

Introduction

BCNAs[a] are a class of compounds with exquisite potency and selectivity against varicella-zoster virus (VZV). In particular, the pentyl phenyl analogue CF1743 (**1**, Figure 1) was found to be active at sub nM concentrations (Table 1), making this compound the most potent and selective nucleoside analogue against VZV to date, while not being toxic for the host cell at the highest possible soluble concentration.[1] The selectivity of **1** depends primarily on a specific phosphorylation by the virus-induced thymidine kinase (VZV-TK), which performs also the second phosphorylation. The host cell-TK1 and TK2 and herpes simplex virus HSV-TK are unable to phosphorylate this nucleoside, explaining the high selectivity of this class of nucleosides.[2] From a clinical viewpoint, **1** offers promise for the treatment of VZV infections. Although it is a thymidine analogue, it was found not to be a good substrate for pyrimidine nucleoside phosphorylase in vitro, [3] but when administered in vivo, some minor cleavage of the glycosidic bond was observed.[4] It is currently unclear whether the in vivo instability is due to the action of prokaryotic pyrimidine nucleoside phosphorylases present in the intestine or to other enzymes present in different organs (i.e., liver). To protect **1** from premature enzymatic degradation by nucleoside phosphorylases or related enzymes, we considered the synthesis of the carbocyclic analogue in which the sugar moiety is replaced by a cyclopentane ring (C-BCNA; **2**), and we hereby report the synthesis and antiviral evaluation of this family of compounds for the first time.

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[a] Abbreviations: BCNA, bicyclic nucleoside analogue; VZV, varicella zoster virus; TK, thymidine kinase; IDU, 5′-iodo-2′-deoxyuridine; DIBAH, diisobutylaluminium hydride; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; BVdU, *E*-5-(2-bromovinyl)-2′-deoxyuridine; ACV, acyclovir/acycloguanosine; HSV, herpes simplex virus.

Chemistry. The carbocyclic analogue of IDU (**12**) needed for the synthesis of C-BCNA (**2**) was performed as described previously.[5] Starting from the commercially available Vince lactam (**3**; Scheme 1), the amido bond was rapidly cleaved under strong acidic conditions, converting **3** into the methyl ester (**4**) in quantitative yield.[6] The protection of the amino group with benzoyl chloride in pyridine was easily achieved, isolating in high yield compound (**5**). The isomerization of **5** into **6** was performed in the presence of DBU in quantitative yield.[6] Reduction of the ester (**6**) with DIBAH, in the presence of one equiv of $AlCl_3$, gave the target allylic alcohol (**7**).[6] The benzylation of compound **7** led to compound **8** and enhanced the steric hindrance around the β-face.[6] The hydroboration was then achieved using the hindered disiamylborane, providing the 3α-hydroxy derivative (**9**).[6] Finally, palladium-catalyzed hydrogenolysis of the benzyl groups afforded the required amino diol (**10**; 41% overall yield).[6] The spectroscopic data are in agreement with those reported.[6]

The synthesis of carba-deoxyuridine (**11**) was achieved, reacting the amino-diol (**10**) with ethoxyacrylisocyanate in the presence of DBU (Scheme 2), affording the urea derivative, which was cyclized in 2 M H_2SO_4 at reflux in good yield (55%).[7] Iodination was performed with molecular iodine in the presence of 1 equiv of ceric ammonium nitrate in acetic acid, obtaining C-IDU (**12**) in good yield (53%;[5] Scheme 2).

Reacting C-IDU (**12**) with ethynyl pentylbenzene under Sonogashira conditions followed by intramolecular cyclization gave in moderate yield (32%) the corresponding carbocycle derivative (**2**).

All spectroscopic and analytical data (NMR, mass, and elemental analysis) confirmed the structure and purity of **2**.

Compound **2** was noted to be chemically stable when treated with 1 M HCl; in fact, after 24 h, no decomposition was observed on TLC (10% methanol in dichloromethane), while for **1**, a complete cleavage of the glycosidic bond occurred in the same conditions after 6 h, observing the formation of the free base of BCNA.

Biological Results. The carbocyclic BCNA (**2**) was evaluated alongside BCNA (**1**) and reference agents BVdU (**13**, Figure

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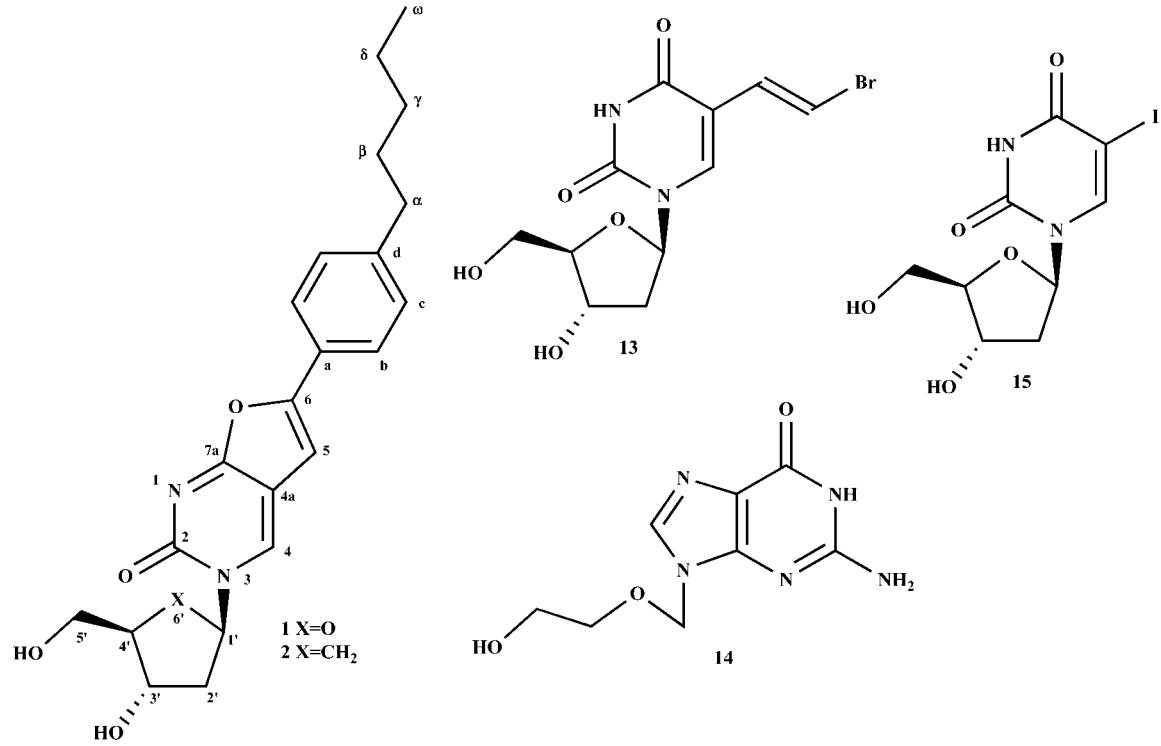


Figure 1. Structures of antiviral nucleosides.

Table 1. Antiviral and Cytostatic Activity of Test Compounds

	EC_{50}[a] (μM) VZV	EC_{50}[a] (μM) VZV	EC_{50}[a] (μM) VZV	EC_{50}[a] (μM) VZV	MCC[b] (μM)	CC_{50}[c] (μM)	IC_{50}[d] VZV-TK (μM)
	TK^+ OKA	TK^+ YS	TK^- 07	TK^- YS	HEL	HEL	
1	0.0003	0.0001	>5	>5	>20	>50	4.9
2	0.49	0.28	>50		>50	>50	44
13	0.005	0.005	>200	>200	>200	>200	1.3
14	2.9	1	74	125	>200	>200	>100

[a] The 50% effective concentration, or compound concentration, required to inhibit VZV-induced cytopathicity by 50%. [b] Minimal inhibitory concentration, or compound concentration, required to cause a microscopically visible alteration of cell morphology. [c] The 50% cytostatic concentration, or compound concentration, required to inhibit HEL cell proliferation by 50%. [d] The 50% inhibitory concentration, or compound concentration, required to inhibit VZV-TK-catalyzed dThd (1 μM) phosphorylation by 50%.

1) and acyclovir (**14**) versus two strains of thymidine kinase-competent VZV and two thymidine kinase-deficient strains (data shown in Table 1). It is clear that replacement of the furanose oxygen of the sugar with a methylene group led to a significant (ca. 1000-fold) loss of activity, making the C-BCNA poorly active against VZV. It is roughly equipotent with acyclovir (**14**) in fact.

It was thought that the interaction between the activating VZV-TK and the carbocycle was perhaps impaired by factors such as the conformation of the sugar or the base. Indeed, it has been demonstrated that the conformation of the sugar ring plays a critical role in determining the affinity of kinases and polymerases for nucleosides.[8] When a nucleoside or nucleotide binds to an enzyme, an imposition of a specific conformation on the sugar ring (north or south) is common for optimal fit that should affect binding energy and catalytic activity. In fact, in the specific case of HSV-TK, it was found that the enzyme prefers nucleosides with the *S*-conformation, where the *N*-nucleosides were found to be inactive or poorly active.[9] Given the high structural similarity of VZV-TK with HSV-TK, it is likely that VZV-TK also prefers the *S*- to the *N*-conformation, as supported by our docking studies.

Conformational Study. A stochastic conformational search was carried out for both **1** and **2**. The predicted lowest energy conformations are shown in Figure 2. The conformation of the sugar appears to be different: compound **1** displays a south conformation, while compound **2** adopts the north one.

NMR Study. The dominant sugar pucker in solution can be identified based on $J_{1'-2'\beta}$ and $J_{3'-4'}$ coupling constants.[10] The percentages of south versus north are calculated from the following equations: $\%S = 100 \times J_{1'-2'\beta}/(J_{1'-2'\beta} + J_{3'-4'})$ and $\%N = 100 - \%S$.

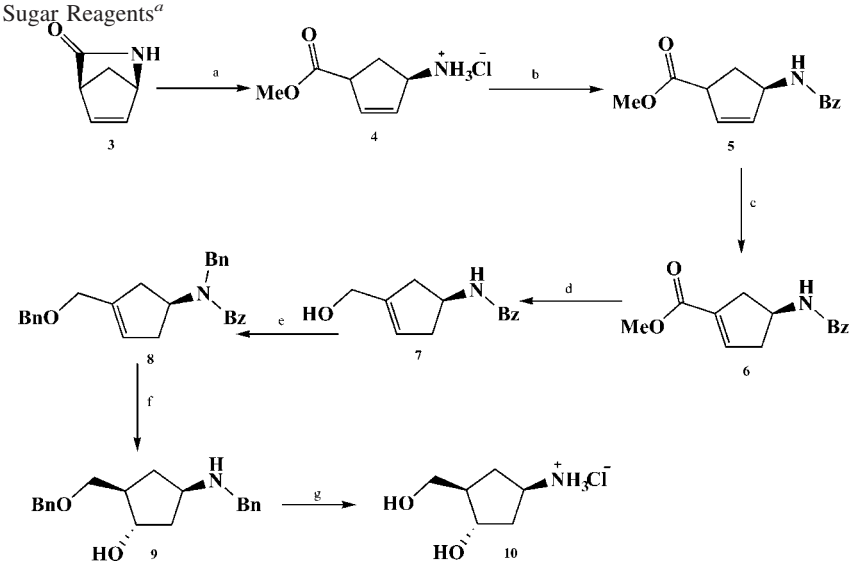
The NMR spectra of compounds **1** and **2** were analyzed and the *J* values required were measured for some antiherpetic nucleosides to obtain the required percentages.

Furthermore, the pseudorotation of **1** and **2** was estimated using the method developed by Altona and Sundarlingam.[10] It was also reported that these compounds do not undergo a significant temperature-dependent conformational change.[11] In fact, when heated from 25 to 37 °C, no significant change in *J* values was observed for compounds **1** and **2**. As it can be seen, the experimental data show that for **1** the preferred conformation is south ($K_{eq} < 1$), with a pseudorotation phase angle $P = 154°$ (150° in the crystal structure of a similar analogue[11]), supporting the hypothesis that VZV-TK prefers this conformation and that **2** adopts the north conformation ($K_{eq} > 1$), which is not tolerated by the VZV enzyme (Table 2).

To further support these data, NOESY spectra were recorded determining also the *syn*/*anti* conformations of the base for both compounds.

The parent (1) shows an *anti*-conformation as the H-4 of the base presents strong interactions with the H-2′$_\beta$. Moreover, medium interactions were observed with the H-5′, OH-5′, and H-3′. Furthermore, the very weak interaction of H-1′ with H-4 supports the *anti*-conformation. From these data it is clear that the H-4 faces above the sugar and that the strong interaction with the H-2′$_\beta$ and the weak interaction with the H-3′ indicates

Scheme 1. Synthesis of Sugar Reagents[a]



[a] Reagents and conditions: (a) MeOH, $SOCl_2$; (b) pyridine, benzoyl chloride; (c) DCM, 1,8-diazabicyclo[5.4.0]undec-7-ene; (d) toluene, DCM, $AlCl_3$, diisobutylaluminiumhydride, HCl, 0°C; (e) *t*-butylmethylether, $Bu_4N^+HSO_3^-$, K_2CO_3, BnBr, NaOH, Δ; (f) THF, Sia_2BH, H_2O_2, NaOH; (g) *i*-PrOH, H_2O, Pd/H_2, HCl.

a predominant south conformation. This result was confirmed also by the analysis of the $J_{H1'\text{-}C2}$ and $J_{H1'\text{-}C6}$,[12,13] obtaining a value of $\chi = -166°$ ($-162°$ in the crystal structure[11]) with an *anti*-population of 76%.

Regarding the carbocycle analogue (**2**), the strong interaction of H-4 with H-1′ supports the *syn*-conformation as no other relevant interactions with the rest of the sugar were detected, implying that the H-4 is far away from the β-face of the sugar. The H-1′ shows the strongest interactions with H-2′ and H-6′; as expected, one strong with the H-4′ and a weak interaction with the H-3′. The H-2′$_\beta$ does not show any interactions with H-4, confirming the *syn*-conformation. The lack of interactions between H-1′ and OH-3′ means that these protons are very distant, and this situation can occur when the sugar is in the north conformation. The quantification of the torsional angle and the *anti*-population could not be calculated, as the long-range coupling constants between the H-1′ and the C-2/C-4 are affected by the presence of the oxygen in the sugar moiety.[13]

The approximate population of the 60° staggered rotamers around the C4′−C5′ bond (ψ) and C5′−O5′ bond (φ) were determined using a Karplus-type equation[14] (Table 3).

Docking Studies. Both compound **1** and compound **2** showed the same docking pose and interactions with residues of the active site, as BVdU (**13**) in its crystallographic pose (Figure 3a). The sugar is in the south conformation, with the 3′-OH forming a H-bond with Tyr66 and the 5′-OH pointing at the ATP binding site. The base is involved in π stacking with Phe63 and Phe136 side chains and a water bridge with Arg143. The pentyl chain is accommodated inside a hydrophobic channel, while the phenyl ring interacts with His97.

Forcing the sugar in *N*-conformation results in the docking pose in Figure 3b, where the hydrogen bond of the 3′-OH is lost and the 5′-OH is not close to the phosphorylation site.

Conclusions

In conclusion, it was thought that a minor change like the replacement of the furanose oxygen of the sugar by a methylene would not greatly affect the shape of the molecule and, hence, the biological activity. Instead, it was found that this substitution deeply affects the molecule, inverting the preferred conformation of the sugar from south to north, as reported for the carbocyclic analogues of deoxyadenine and deoxyguanosine.[15] Moreover, this switch also changed the conformation of the base in solution from *anti* to *syn*, making this compound a markedly poorer substrate for the VZV-TK. This is indeed supported by the VZV-TK inhibition data: compound **1** inhibits the VZV-TK at 4 μM, while **2** is only inhibitory at 44 μM (Table 1). Although the preferred conformation of **2** in solution is north, the low energetic barrier to switch from north to south[8] may allow the VZV-TK to phosphorylate the compound.

It should be noticed that VZV-TK acts as the activating (phosphorylating) enzyme of the BCNA, but the eventual target of the BCNA for antiviral activity is currently unknown. Therefore, a lesser affinity of the phosphorylated BCNA for its antiviral target may also be an additional reason for the markedly lower antiviral activity of the compound.

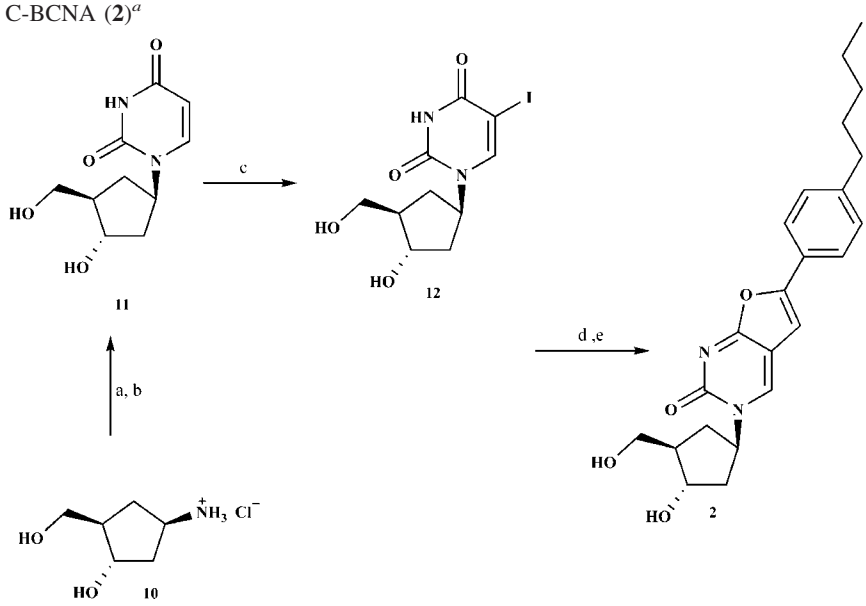
Thus, although the glycosidic bond stability of **2** is most likely greatly enhanced over **1**, the greatly reduced antiviral activity of the carbocycle makes it of limited interest for development. It may be that phosphate prodrug (ProTide) strategies may address this, and these are now under investigation.

Experimental Section

Antiviral Assays. Confluent HEL cells grown in 96-well microtiter plates were inoculated with VZV at an input of 20 PFU (plaque forming units) per well. After a 1–2 h incubation period, residual virus was removed and the infected cells were further incubated with MEM (supplemented with 2% inactivated FCS, 1% L-glutamine, and 0.3% sodium bicarbonate) containing varying concentrations of the compounds. Antiviral activity was expressed as EC_{50} (50% effective concentration), the compound concentration required to reduce viral plaque formation after 5 days (VZV) by 50% compared to the untreated control.

Cytotoxicity Assays. Confluent monolayers of HEL cells as well as growing HEL cells in 96-well microtiter plates were treated with different concentrations of the experimental drugs. Cell cultures were incubated for 3 (growing cells) or 5 (confluent cells) days. At day 3, the cells were trypsinized and the cell number was determined using a Coulter counter. The 50% cytostatic concentration (CC_{50}) was defined as the compound concentration required to reduce the cell number by 50%. At day 5, microscopically visible

Scheme 2. Synthesis of C-BCNA (**2**)[a]



[a] Reagents and conditions: (a) DMF, 1,8-diazabicyclo[5.4.0]undec-7-ene, 0.5 M ethoxyacrylisocyanate in benzene; (b) 2 M H_2SO_4, Δ; (c) CH_3COOH, ceric ammonium nitrate, I_2, Δ; (d) DMF, 4-ethynylpentylbenzene, $Pd(Ph_3)_4$, CuI, diisopropylethylamine; (e) CuI, triethylamine, Δ.

morphological changes were recorded and the minimal cytotoxic concentration (MCC) was determined.

Enzyme Reaction Assay. The IC_{50} of the test compounds against phosphorylation of 1 μM [CH_3-^{3}H]dThd as the natural substrate by VZV-TK was determined under the following reaction conditions: the standard reaction mixture (50 μL) contained 50 mM Tris-HCl, pH 8.0, 2.5 mM $MgCl_2$, 10 mM dithiothreitol, 2.5 mM ATP, 10 mM NaF, 1.0 mg/mL bovine serum albumin, 1 μL [CH_3-^{3}H]dThd (0.1 μCi), an appropriate amount of test compound, and 5 μL of Milli-Q water. The reaction was started by the addition of purified recombinant enzyme (7.75 ng) and then incubated at 37 °C for 30 min, and the reaction was terminated by spotting an aliquot of 45 μL onto DE-81 discs (Whatman, Maidstone, England). After 15 min, the discs were washed three times for 5 min each in 1 mM $HCOONH_4$ while shaking, followed by 5 min in ethanol (70%). Finally, the filters were dried and assayed for radioactivity. The IC_{50} was defined as the drug concentration required to inhibit thymidine phosphorylation by 50%.

Stability Assay. Compound **1** (10 mg) was dissolved in a mixture of MeOH (5 mL) and $HCl_{(c)}$ (0.45 mL). The reaction was monitored by TLC (10% MeOH in DCM) every hour, showing the total cleavage of the glycosidic bond after 6 h. Compound **2** (10 mg) was dissolved in a mixture of MeOH (5 mL) and $HCl_{(c)}$ (0.45 mL). The reaction was monitored by TLC (10% MeOH in DCM) every hour. After 24 h, no detectable cleavage was observed. R_f (**1**), 0.43; R_f (**2**), 0.34; R_f (free base), 0.50.

Solvents and Reagents. The following anhydrous solvents with a sureseal stopper and IDU were bought from Aldrich: methanol (MeOH), *t*-butylmethyl ether, dichloromethane (DCM), diethyl ether (Et_2O), *N*,*N*-dimethylformamide (DMF), pyridine (Pyr), tetrahydrofuran (THF), triethylamine (TEA), and toluene. All reagents commercially available were used without further purification. Compounds **1** and **12** were synthesized according to the procedures previously described.[1,16]

Thin Layer Chromatography (TLC). Precoated, aluminum-backed plates (60 F_{254}, 0.2 mm thickness, Merck) were visualized under both short (254 nm) and long wave (365 nm) ultraviolet light.

Column Chromatography (CC). Column chromatography processes were carried out using silica gel supplied by Fluka (silica gel 60, 35–70 μm, 220–440 mesh). Glass columns were slurry packed using the appropriate eluent, and samples were applied either as a concentrated solution in the same eluent or preadsorbed on silica gel.

High Performance Liquid Chromatography (HPLC). Analytical procedures were run on a Varian ProStar instrument (LC work station, Varian Prostar 355 LC detector) using a Polaris C18-A 10 μ column; elution was performed using a mobile phase consisting of water/acetonitrile in gradient (water 100% to 0% in 20 min).

Nuclear Magnetic Resonance (NMR). ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorder on a Bruker Avance 500 spectrometer at 25 °C. Spectra were calibrated to the residual signal of the deuterated solvent used. ^{13}C NMR spectra were proton-decoupled. Chemical shifts are given in parts per million (ppm) and coupling constants (*J*) in Hertz. The following abbreviations were used: s (singlet), d (doublet), t (triplet), q (quartet), qn (quintet), sx (sextet), m (multiplet), bs (broad signal), dd (doublet of doublets), dt (doublet of triplets), dq (doublet of quartets), tt (triplet of triplets), ddd (doublet of doublets of doublets), ddt (doublets of doublets of triplet). The assignment of the signals was performed using 2D-NMR experiments COSY, HSQC, and NOESY. For compounds **1** and **2**, a line broadening of −2 Hz and Gaussian Bell in the range 0.2–0.3 was used to measure the coupling constants on ^{1}H NMR. The coupling constants obtained were used to simulate the spectra using Bruker NMRSim 4.6 (Bruker TopSpin 2.0) and then compared with the recorded spectra. The images are available in Supporting Information.

Mass Spectroscopy (MS). Mass spectra were recorded on a microTOFLC Bruker Daltonics spectrometer.

Elemental Analysis (CHN). Elemental microanalysis was performed as a service by the School of Pharmacy at the University of London.

Molecular Modeling. Energy minimization and conformational analysis were carried out using MMFF94x forcefield, as implemented in MOE 2006.08 Chemical Computing Group, Inc. (Montreal, Quebec, Canada).

All ligand structures were docked using Plants v-1.06[17] inside the VZV-TK structure in complex with BVdU (PDB code 1osn). The docking site was limited inside a 12 Å radius sphere centered in the mass center of the crystallized ligand. One water molecule contacting the *C*2 carbonyl was kept in the simulations. The alignment of the target ligand with the crystallographic coordinates of BVdU suggested that the pentyl chain could be accommodated inside a lipophilic channel/pocket not contacted by BVdU. Molecular docking failed to reproduce this pose due to steric clashes. However, after local minimization of the side chains of the amino acids surrounding the pocket, compounds **1** and **2** were successfully docked.

***cis*-4-Amino-2-cyclopentenecarboxylic Acid Methyl Ester Hydrochloride (4).** $SOCl_2$ (8 mL) was slowly added to MeOH (50 mL) at 0 °C, then 5.45 g (50 mmol) of Vince lactame **3** was

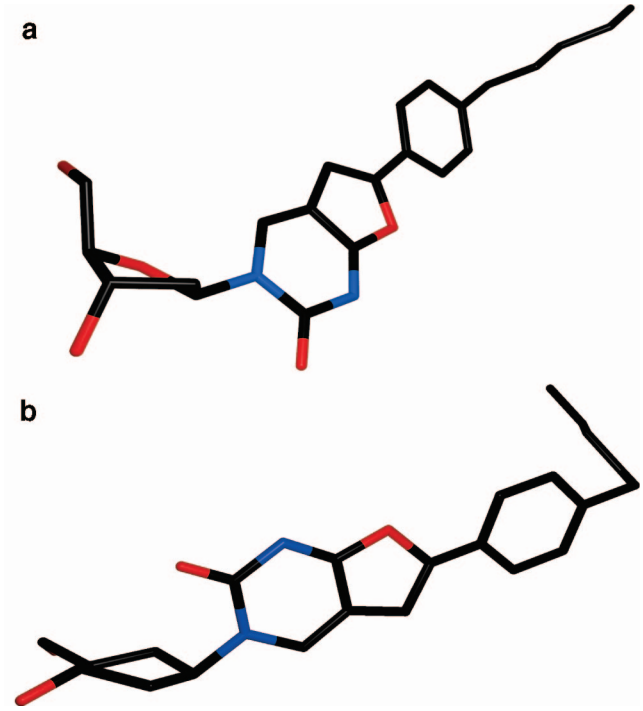


Figure 2. (a) BCNA (**1**) in the south conformation. (b) C-BCNA (**2**) in north conformation.

Table 2. North and South Conformation of the Test Compounds

	%N	*%S*	K_{eq} (*N*/*S*)	$J_{1'-2'\beta}$ (Hz)	$J_{3'-4'}$ (Hz)
(**1**) BCNA	39	61	0.64	6.10	3.90
(**2**) C-BCNA	68	32	2.12	3.52	7.48
(**13**) BVdU	36	64	0.56	6.57	3.66
(**15**) IDU	33	67	0.49	6.68	3.22

Table 3. Conformational Parameters of the Test Compounds

	(**1**) BCNA	(**2**) C-BCNA
Glycosidic Bond		
anti-population %	76	N/A
χ (O4′−C1′−N1−*C2*)	−166°	*syn*
Sugar Pucker		
phase angle *P*	154°	50°
pucker amplitude τ_m	42°	40°
C4′−C5′		
$\%\psi_-$	23	34
$\%\psi_a$	15	31
$\%\psi_+$	62	35
C5′−O5′		
$\%\varphi_-$	33	26
$\%\varphi_a$	33	48
$\%\varphi_+$	34	26

added and the mixture was stirred for 2 h at the same temperature. The solvent was removed under reduced pressure to obtain 8.88 g (100%) of the title compound as a white solid. ^{1}H NMR (DMSO): δ 8.44 (3H, bs, NH_3), 6.07 (1H, dd, J = 5.5 Hz 2.2 Hz, H-2), 5.90 (1H, dd, J = 5.5 Hz, 2.4 Hz, H-3), 4.21–4.12 (1H, m, H-1), 3.72–3.67 (1H, m, H-4), 3.65 (3H, s, OCH_3), 2.60–2.52 (1H, m, H-6), 2.00–1.92 (1H, m, H-6).

***cis*-4-Benzoylamino-2-cyclopentenecarboxylic Acid Methyl Ester (5).** Compound **4** (8.8 g, 50 mmol) was dissolved in pyridine (50 mL), and benzoyl chloride (6.4 mL, 1.1 equiv) was added dropwise at 0 °C. The mixture was then stirred at room temperature for 3 h, after which it was poured into ice. When the ice completely melted, the precipitate was filtered, washed with water, and dried under vaccum to obtain 11.04 g (90%) of the title compound as a white solid. ^{1}H NMR (DMSO): δ 8.53 (1H, d, J = 7.0 Hz, NH), 7.88 (2H, d, J = 7.5 Hz, Ph-H_2), 7.52 (1H, t, J = 7.5 Hz, Ph-H_4), 7.45 (2H, t, J = 7.5 Hz, Ph-H_3), 5.94–5.90 (1H, m, H-2), 5.90–5.85 (1H, m, H-3), 5.03 (1H, q, J = 7.8 Hz, H-1), 3.66 (3H, s, OCH_3), 3.64–3.59 (1H, m, H-4), 2.58 (1H, dt, J = 13.0 Hz 7.8 Hz, H-6), 1.94 (1H, dt, J = 13.0 Hz 7.8 Hz, H-6).

4-(Benzoylamino)-1-cyclopentenecarboxylic Acid Methyl Ester (6). Compound **5** (11.04 g, 45 mmol) was dissolved in DCM (55 mL) and DBU (9.6 mL, 1.45 equiv) was added. The mixture was stirred at room temperature for 15 h, after which it was cooled at 0 °C, and ice (17 g), H_2O (11 mL), and H_2SO_{4CAN} (3.3 mL) were added. The organic phase was separated and washed with water, dried over $MgSO_4$, and evaporated under reduced pressure. The resulting solid was triturated with Et_2O, filtered under vacuum, and dried to obtain 11 g (100%) of the title compound as a white solid. ^{1}H NMR (DMSO): δ 8.60 (1H, d, J = 6.2 Hz, NH), 7.86 (2H, d, J = 7.5 Hz, Ph-H_2), 7.52 (1H, t, J = 7.5 Hz, Ph-H_4), 7.45 (2H, t, J = 7.5 Hz, Ph-H_3), 6.76–6.70 (1H, m, H-3), 4.66–4.58 (1H, m, H-1), 3.69 (3H, s, OCH_3), 2.95–2.84 (2H, m, H-2 + H-6), 2.61–2.49 (2H, m, H-2 + H-6).

***N*-[3-(Hydroxymethyl)-3-cyclopentenyl]-benzamide (7).** Compound **6** (11 g, 45 mmol) was suspended in a mixture of DCM (50 mL) and toluene (25 mL), and the suspension was cooled at 0 °C. $AlCl_3$ (6 g, 1 equiv) was added, and the reaction mixture was stirred for 20 min to give a cloudy solution. The resultant mixture was then cooled to 0 °C and a 25% solution of DIBAH in toluene (69 mL, 2 equiv) was added at a rate such that the reaction mixture remained in the temperature range of 0–10 °C. Upon completion of the addition of DIBAH, the reaction mixture was stirred for 10 min at 0 °C. After this time, the mixture was slowly added to a solution of 4 M HCl at room temperature. Toluene was added and stirring was continued for a further 30 min. The reaction mixture was the cooled to 0 °C and stirred at this temperature for 1 h. The product was harvested by filtration and washed with toluene, 2 M HCl. and H_2O and dried under vacuum to yield the title compound (8.9 g, 90%) as a white solid. ^{1}H NMR (DMSO): δ 8.50 (1H, d, J = 6.8 Hz, NH), 7.85 (2H, d, J = 7.8 Hz, Ph-H_2), 7.51 (1H, t, J = 7.8 Hz, Ph-H_4), 7.45 (2H, t, J = 7.8 Hz, Ph-H_3), 5.52–5.48 (1H, m, H-3), 4.58 (1H, sx, J = 6.8 Hz, H-1), 4.02–3.93 (2H, m, H-5), 2.72–2.57 (2H, m, H-2 + H-6), 2.39–2.25 (2H, m, H-2 + H-6).

***N*-(Benzyl)-*N*-[(3-benzyloxymethyl)-3-cyclopentenyl]-benzamide (8).** Compound **7** (8.9 g, 41 mmol) was added to *t*-butyl methyl ether (90 mL), and the resultant suspension stirred at room temperature while potassium carbonate (34 g), tetra-*n*-butylammonium hydrogen sulfate (2.1 g), and sodium hydroxide (11.5 g) were added sequentially. Stirring was continued while the internal temperature was raised to reflux via a hot water bath. Reflux was continued for a further 25 min, and then benzyl bromide (10.7 mL, 2.2 equiv) was added dropwise to the refluxing suspension. Reflux was continued for a further 160 min. Heating was discontinued and methanol (1 mL) was added over 30 s. The mixture was then cooled, and distilled water (5×, 18 mL) was added over 4 min. The mixture was further cooled with a cold water bath, transferred to a glass separating flask, and the lower aqueous phase was removed. The aqueous phase was extracted with *t*-butyl methyl ether, and the organic phases were combined and washed with distilled water. The organic phase was dried and the residue was purified by column chromatography (hexane/ethyl acetate 9:1) to obtain the title compound (14.05 g, 86%) as a yellow oil. ^{1}H NMR (DMSO): δ 7.55–7.21 (15H, m, Ph + 2 × Bn), 5.53 (1H, bs, H-3), 4.62 (3H, Bs, Bn + H-1), 4.38 (2H, s, Bn), 3.90 (2H, s, H-5), 2.40 (4H, bs, H-2 + H-6). ^{13}C NMR (DMSO): δ 172.21 (CO), 141.74 (C-4), 140.26, 138.98, 131.14, 131.01, 130.43, 130.21, 130.10, 129.59, 129.37, 129.18, 128.49, 128.16 (Ar), 126.59 (C-3), 73.13 (Bn-CH_2), 69.96 (C-5), 60.00 (bs, C-1), 46.62 (Bn-CH_2), 38.86 (bs, *C*2), 38.42 (bs, C6).

4-Benzylamino-2-benzyloxymethyl-1-cyclopentanol (9). 2-Methyl-2-butene (25 mL) was added to tetrahydrofuran (25 mL), and the resulting solution was stirred under nitrogen and cooled to 0 °C. Borane dimethylsulphide complex (10.6 mL) was then added at a rate such that the temperature of the mixture did not exceed

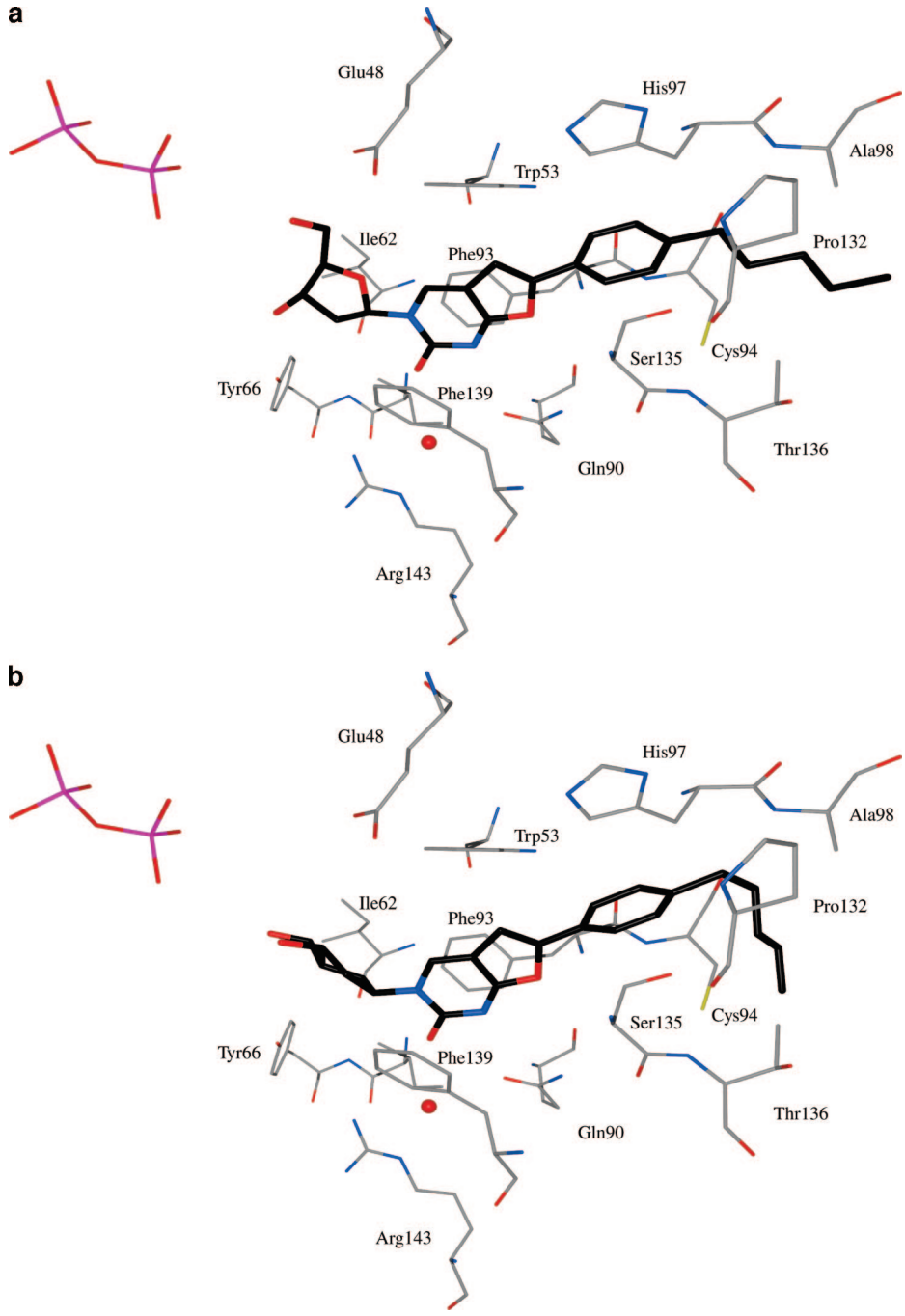


Figure 3. (a) BCNA (**1**) docked in the south conformation inside VZV-TK with α,β-phosphate of ADP. (b) C-BCNA (**2**) docked in north conformation inside VZV-TK with α,β-phosphate of ADP.

24 °C. This solution was then stirred and treated with a solution of **8** (14.05 g, 35 mmol) in tetrahydrofuran (28 mL) over 40 min. Stirring was continued for 24 h at room temperature and then the reaction was quenched by cooling the solution to 0 °C and adding dropwise a mixture of water (1.5 mL) and tetrahydrofuran (14 mL). The solution was recooled to 0 °C and then 3 M sodium hydroxide (35 mL) was added over 12 min. The solution/emulsion obtained was cooled to −20 °C and hydrogen peroxide (30%, 36 mL) was added dropwise over about 2 h, such that an internal temperature of <30 °C was maintained. After 20 min, a solution of sodium sulfite (7 g) in water (28 mL) was added over 70 min. Following the final addition of hydrogen peroxide, the mixture was left under nitrogen overnight. The mixture was then stirred and methyl isobutyl ketone (70 mL) was added. The phases were separated and the aqueous phase was extracted with methyl isobutyl ketone. The combined organic phases were washed with water and the organic phase was reduced to about a 70 mL volume. The resulting yellow solution was stirred and cooled to 4 °C and then concentrated hydrochloric acid (3 mL) was added. The cooling bath was removed, and after 30 min, *t*-butyl methyl ether (70 mL) was added dropwise. The pH was lowered to 1–2 during the addition of *t*-butyl methyl ether by the further addition of concentrated hydrochloric acid. The resulting suspension was stirred for about 1 h with ice/water cooling and then filtered. The residual precipitate was washed with *t*-butyl methyl ether and then dried to give the title compound as a white solid (7.5 g, 63%). ^{1}H NMR (DMSO): δ 9.6 (2H, bs, NH_2), 7.7–7.2 (10H, m, 2 × Bn), 4.90 (1H, br, OH-3), 4.48 (2H, m, Bn), 4.10 (2H, m, Bn), 3.98 (1H, m, H-3), 3.62–3.53 (1H, m, H-1), 3.53–3.35 (2H, m, H-5), 2.30–2.20 (1H, m, H-6), 2.10–1.82 (3H, m, H-2 + H-4), 1.54–1.47 (1H, m, H-6).

4-Amino-2-hydroxymethyl-1-cyctopentanol (10). Compound **9** (5 g, 16 mmol) was dissolved in isopropanol (12.5 mL) and water (4 mL). Charcoal (2.5 g) was added, and the mixture was stirred for 30 min. After this time, celite (1.25 g) was added and the mixture

was filtered through a bed of celite. The filter bed was washed with a mixture of isopropanol (6 mL) and water (2 mL) twice. The filtrate and washes were combined. Palladium (5%) on charcoal (5 g) was charged into a dry flask. The flask was purged with nitrogen, and the combined filtrate and washes from above were added. The resulting mixture was stirred and heated to 50 °C under an atmosphere of hydrogen for 3 h. After this time, the reaction mixture was allowed to cool and then filtered through a pad of celite. The filter pad was then washed with mixtures of isopropanol and water. The combined filtrate and washings were evaporated to approximately 20 mL. *n*-Butanol (10 mL) was added, and the mixture was re-evaporated under reduced pressure to 3 mL. Methanol (2 mL) containing concentrated hydrochloric acid (2 mL) was added followed by *n*-butanol (10 mL). The solvent was removed under reduced pressure to yield a gum. This was taken up in methanol, and the volume reduced under pressure to the point at which precipitation had just begun. Acetone (10 mL) was added slowly, and filtration followed by washing with *n*-butanol/acetone (1:1) and acetone yielded, after drying under reduced pressure, the title compound as a white solid (2.01 g, 95%). ^{1}H NMR (DMSO): δ 8.20 (3H, bs, NH_3), 4.90–4.50 (2H, m, bs, OH-3 + OH-5), 3.94 (1H, m, H-3), 3.53 (1H, m, H-1), 3.50–3.25 (2H, m, H-5), 2.13 (1H, m, H-2), 1.95–1.70 (3H, m, H-6 + H-4), 1.34 (1H, m, H-2).

1-(3-Hydroxy-4-(hydroxymethyl)cyclopentyl)uracil (11). To a solution of **10** (2.01 g, 12 mmol) in dimethylformamide (15 mL) containing DBU (2 mL, 12 mmol) and 4 Å molecular sieves (3 g) at −20 °C was added a 0.5 M benzenic solution of ethoxyacryl-isocyanate (37 mL, 1.7 equiv). The mixture was stirred at −15 °C for 1 h and then left to warm to room temperature. After 18 h, the mixture was filtered and the filtrate was evaporated. The residue was purified by column chromatography on silica gel, eluting with chloroform−methanol (9: 1), to give the intermediate acryloylurea. A solution of the acryloylurea thus obtained (2.48 g, 9.1 mmol) in 5% sulfuric acid (50 mL) was refluxed for 3 h. The mixture was cooled to room temperature and adjusted to pH 7 with 2 N sodium hydroxide. The solution was evaporated under reduced pressure and the residue was dried and then extracted with ethanol. The combined ethanolic solution was evaporated under reduced pressure and purified by column chromatography ($CHCl_3$/MeOH 9:1) to give 1.48 g (72%) of the title compound as a white solid. ^{1}H NMR (DMSO): δ 10.30 (1H, bs, NH), 7.70 (1H, d, J = 7.9 Hz, H-5), 5.56 (1H, d, J = 7.9 Hz, H-6), 4.94 (1H, qn, J = 8.9 Hz, H-1′), 4.83 (1H, bs, OH-3′), 4.71 (1H, bs, OH-5′), 4.09–4.02 (1H, m, H-3′), 3.51–3.35 (2H, m, H-5′), 2.12–2.00 (1H, m, H-2′), 1.95–1.72 (3H, m, H + 4′ + H-6′), 1.43–1.34 (1H, m, H-2′).

1-(3-Hydroxy-4-(hydroxymethyl)cyclopentyl)-5-iodo-uracil (12). To solution of **11** (1.48 g, 6.5 mmol) in glacial AcOH was added I_2 (0.83 g, 0.5 equiv) and CAN (1.78 g, 0.5 equiv), and the mixture was then stirred at 80 °C for 1 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography ($CHCl_3$/MeOH 9:1) to afford 1.21 g (53%) of the titled compound as a yellow solid. ^{1}H NMR (DMSO): δ 9.51 (1H, bs, NH), 9.51 (1H, s, H-6), 4.96–4.87 (1H, m, H-1′), 4.71 (1H, bs, OH-3′), 4.82 (1H, bs, OH-3′), 4.60 (1H, bs, OH-5′), 4.10–3.95 (1H, m, H-3′), 3.43–3.29 (2H, m, H-5′), 2.11–2.00 (1H, m, H-2′), 1.87–1.68 (3H, m, H + 4′ + H-6′), 1.48–1.41 (1H, m, H-2′).

3-(3-Hydroxy-4-(hydroxymethyl)cyclopentyl)-6-(4-pentylphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one (2). To a stirred solution of **12** (1.06 g, 3 mmol) in dry DMF (15 m) at room temperature, 4-ethynylpentylbenzene (1.55 g, 3 equiv), tetrakis(triphenylphosphine)Pd^0 (0.35 g, 0.1 equiv), copper(I) iodide (0.12 g, 0.2 equiv), and DIPEA (1 mL, 2 equiv) were added. The reaction mixture was stirred for 15 h at room temperature, after which time, triethylamine (15 mL) and further copper(I) iodide (0.12 g, 0.2 equiv) were added. The reaction mixture was then heated at 80 °C and stirred for 4–6 h. The solvent was removed under reduced pressure, and the resulting residue was purified by column chromatography ($CHCl_3$/MeOH 95:5) to afford 0.38 g (32%) of the title compound as a light brown solid. A small portion (100 mg) was further purified by preparative HPLC (H_2O/ACN 0:100 in 20 min) to afford 40 mg of pure compound as white solid. ^{1}H NMR (DMSO): δ 8.65 (1H, s, H-4), 7.74 (2H, d, J = 8.1 Hz, Ph_b), 7.32 (2H, d, J = 8.1 Hz, Ph_c), 7.22 (1H, s, H-5), 5.21 (1H, tt, J = 7.5 Hz, 3.5 Hz, H-1′), 4.79 (1H, d, J = 4.1 Hz, OH-3′), 4.64 (1H, t, J = 4.7 Hz, OH-5′), 4.06 (1H, ddt, J = 7.5 Hz, 6.0 Hz 4.1 Hz, H-3′), 3.54 (1H, ddd, J = 10.5 Hz 5.3 Hz, 4.7 Hz, H-5′$_a$), 3.44 (1H, ddd, J = 10.5 Hz, 6.0 Hz, 4.7 Hz H-5′$_b$), 2.62 (2H, t, J = 7.4 Hz, $CH_2\alpha$), 2.26 (1H, dt, J = 12.6 Hz, 7.5 Hz, H-6′$_\alpha$), 2.06 (1H, ddd, J = 12.9 Hz, 6.0 Hz, 3.5 Hz, H-2′$_\beta$), 2.01–1.92 (1H, ddd, J = 12.9 Hz, 6.0 Hz, 7.5 Hz, H-2′$_\beta$) 1.98–195 (1H, m, H-4′), 1.59 (2H, qn, J = 7.7 Hz, $CH_2\beta$), 1.51 (1H, ddd, J = 12.6 Hz, 9.4 Hz, 3.5 Hz, H-6′$_\beta$), 1.36–1.22 (4H, m, $CH_2\gamma$ + $CH_2\delta$), 0.87 (3H, t, J = 6.8 Hz, $CH_3\omega$). MS: 419 (M + Na).

3-(2-Deoxy-β-D-ribofuranosyl)-6-(4-pentylphenyl)furo[2,3-*d*]pyrimidin-2(3*H*)-one (1). ^{1}H NMR (DMSO): δ 8.84 (1H, s, H-4), 7.75 (2H, d, J = 7.6 Hz, Ph_b), 7.33 (2H, d, J = 7.6 Hz, Ph_c), 6.20 (1H, t, J = Hz 6.1 Hz, H-1′), 5.28 (1H, d, J = 4.2 Hz, OH-3′), 5.15 (1H, t, J = 5.1 Hz, OH-5′), 4.27 (1H, dq, J = 6.1 Hz, 4.2 Hz, H-3′), 3.94 (1H, q, J = 3.9 Hz, H-4′), 3.72 (1H, ddd, J = 12.1 Hz, 5.1 Hz, 3.9 Hz, H-5′$_a$), 3.65 (1H, ddd, J = 12.1 Hz, 5.1 Hz, 4.2 Hz, H-5′$_b$), 2.61 (2H, t, J = 7.5 Hz, $CH_2\alpha$), 2.42 (1H, ddd, J = 13.5 Hz, 6.3 Hz, 4.1 Hz, H-2′$_\alpha$), 2.11 (1H, dt, J = 13.5 Hz, 6.1 Hz, H-2′$_\beta$), 1.59 (2H, qn, J = 7.5 Hz, $CH_2\beta$), 1.35–1.24 (4H, m, $CH_2\gamma$ + $CH_2\delta$), 0.86 (3H, t, J = 7.1 Hz, $CH_3\omega$).

5-(*E*)-(2-Bromovinyl)-2′-deoxyuridine (BVdU; 13). ^{1}H NMR (DMSO): 11.55 (1H, s, NH), 8.08 (1H, s, H-6), 7.26 (1H, d, J = 13.4 Hz, H-5$_b$), 6.85 (1H, d, J = 13.4 Hz, H-5$_a$), 6.13 (1H, t, J = 6.6 Hz, H-1′), 5.05 (2H, bs, OH-3′ + OH-5′), 4.25 (1H, dt, J = 4.8 Hz, 3.7 Hz, H-3′), 3.79 (1H, q, J = 3.7 Hz, H-4′), 3.64 (1H, dd, J = 12.0 Hz, 3.7 Hz, H-5′$_a$), 3.57 (1H, dd, J = 12.0 Hz, 3.7 Hz, H-5$_b$), 2.14 (2H, dd, J = 6.6 Hz, 4.8 Hz, H-2′).

5-Iodo-2′-deoxyuridine (IDU; 15). ^{1}H NMR (DMSO): 11.66 (1H, s, NH), 8.39 (1H, s, H-6), 6.10 (1H, t, J = 6.6 Hz, H-1′), 5.23 (1H, d, J = 4.4 Hz, OH-3′), 5.13 (1H, t, J = 4.8 Hz, OH-5′), 4.24 (1H, dq, J = 4.4 Hz, 3.2 Hz, H-3′), 3.80 (1H, q, J = 3.2 Hz, H-4′), 3.63 (1H, ddd, J = 12.0 Hz, 4.8 Hz, 3.2 Hz, H-5′$_a$), 3.57 (1H, ddd, J = 12.0 Hz, 4.8 Hz, 3.2 Hz, H-5$_b$), 2.17–2.09 (2H, m, H-2′).

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Supporting Information Available: Elemental analysis of the final compound **2** of this study. ^{13}C NMR of compounds **2**, **4**, **5**, **6**, **7**, **8**, **9**, **10**, **11**, and **12**. NMR images of compounds **1** and **2**: ^{1}H NMR, ^{13}C pendant, COSY, HSQC, NOESY, and NOE at H-4. Image simulations of the spin systems of the sugar moiety of compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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