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# 2′-Fluorosugar analogues of the highly potent anti-varicella-zoster virus bicyclic nucleoside analogue (BCNA) Cf 1743

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ABSTRACT

We report the preparation of 2′-α-F, 2′-β-F and 2′,2′-difluoro analogues of the leading anti-varicella zoster virus (VZV) pentylphenyl BCNA Cf 1743. VZV thymidine kinase showed the highest phosphorylating capacity for the β-fluoro derivative, that retained equal antiviral potency as the parent compound. In contrast, the α-fluoro- and 2′,2′-difluoro BCNA derivatives were markedly less (~100-fold) antivirally active.


In 1999 we first reported the anti-VZV activity of the bicyclic nucleoside analogue family now known as the BCNAs.[1] We subsequently reported nanomolar activity and exclusive anti-VZV selectivity for the pentylphenyl BCNA (Fig. 1, **1**).[2]

As its 5′-valyl Pro-Drug FV100 (Fig. 1, **2**) human phase 2 clinical trials for VZV shingles have recently commenced.[3]

We have reported extensively on the structure–activity relationships surrounding this family of potent antivirals.[4] In general, there is little tolerance for structural modifications; indeed we recently reported that the corresponding carbocycle, a modification often tolerated amongst antiviral nucleosides, is very poorly active in this case.[5] Also, the arabinosyl BCNA was considerably less inhibitory than its parental 2′-deoxyribosyl BCNA.[6]

There have been a number of cases where 2′-modification, in particular 2′-fluorination of bioactive nucleosides leads to enhancements in the biological activity profile, notably, the anti-cancer agent gemcitabine with a 2′,2′-difluoro pattern[7] and Pharmasset's anti-HCV agent PSI6130 (2′-deoxy-2′-α-fluoro-2′-β-C-methyl cytidine).[8] Therefore, we were interested to prepare various 2′-fluorinated analogues of the parent BCNA (**1**) (Fig. 2).

The desired 2′-fluoro-substituted BCNAs were prepared via sugar base coupling of protected 5-iodouracil to the appropriate fluoro sugar followed by construction of the BCNA base.

Thus, as shown in Scheme 1, 5-iodouracil (**3**) was silylated to give (**4**) and 1,3,5-tri-*O*-benzoyl-2-deoxy-2-β-fluororibose (**5**) was converted to its 1-bromo analogue (**6**) using HBr/AcOH,[9] and these reagents were allowed to couple using HMDS and ammonium sulfate to give mixed anomers of the protected nucleoside (**7**).[9]

The two isomers were separated by filtration to give the pure β-anomer as a white solid **7** in a yield of 51%. The deprotection of the benzoyl groups was performed using sodium methoxide and the corresponding unprotected compound **8** converted to its *p*-pentylphenyl BCNA (**9**) by standard methods[1,2] in a yield of 45% from (**7**). Compound (**9**) showed spectroscopic ($^1$H NMR and $^{13}$C NMR) and analytical data entirely as expected including a $^{19}$F NMR peak at −198 ppm and other data (high resolution mass spectra and HPLC) confirmed its structure and purity.[10] Similarly prepared by analogous methods were the α-fluoro analogue (**10**) and the 2′,2′-difluoro BCNA derivative (**11**). Compound (**10**) was prepared starting from the commercial available 2′-α-F-2′-deoxyuridine which was iodinated at the 5-position and then coupled with the phenyl acetylene under standard method. Compound (**11**) was prepared starting from commercially available 2-deoxy-

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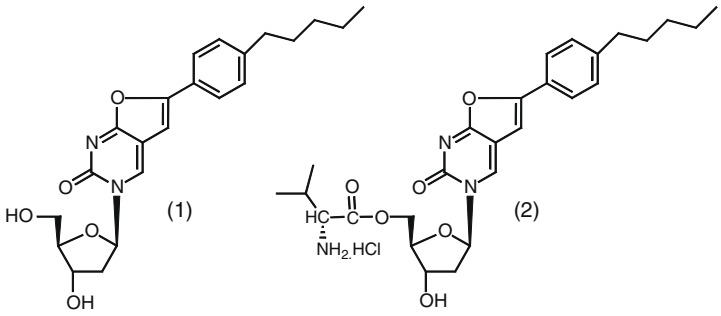



**Figure 1.**

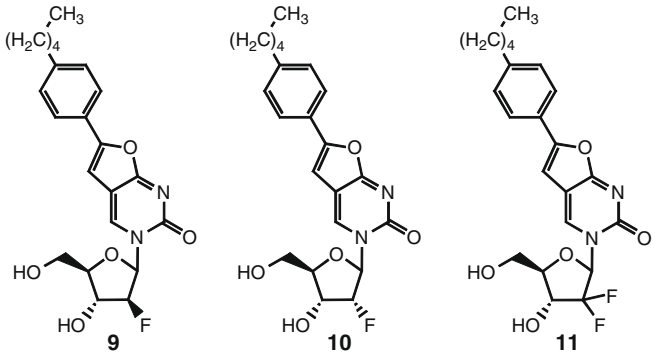



**Figure 2.**

2,2-difluoro-D-*erythro*-pentafuranos-1-ulose-3,5-dibenzoate which was reduced to benzoylated lactol using LiAl(O-*t*Bu)$_3$H and then mesylated. The crude mesylate was coupled with silyl-protected 5-iodouracil in dichloroethane and the β-anomer was obtained by precipitation from the organic solvent after the work-up. Deprotection of the benzoyl groups by sodium methoxide in methanol provided the desired nucleoside.

Given the crucial requirement for the BCNAs to be 5′-phosphorylated by VZV thymidine kinase (TK) for their antiviral activity[11] we first probed their interaction with VZV TK.[12] Thus, in Table 1 we show the 50% inhibitory concentrations ($IC_{50}$) of (**1**) and (**9–11**) for the phosphorylation of 1 μM [$CH_3$-$^3$H]thymidine by purified recombinant VZV TK.

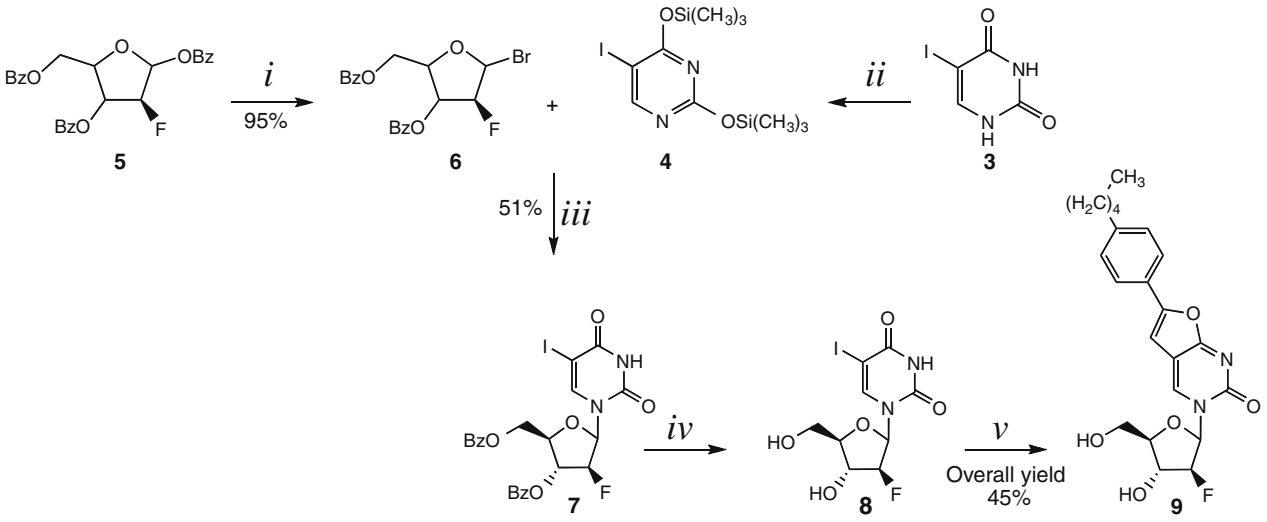
**Table 1**

| Compound | 2′-Fluoro position | VZVTK $IC_{50}$/μM |
|---|---|---|
| **1** | — | 3.3 |
| **9** | β-Fluoro | 38 |
| **10** | α-Fluoro | 2.7 |
| **11** | Difluoro | ~13 |

It is notable from Table 1 that the α-fluoro analogue (**10**) retains low μM potency as an inhibitor of VZV TK-catalysed dThd phosphorylation. The β-fluoro derivative **9** was at least 10-fold less inhibitory. The 2′,2′-difluoro analogue **11** showed an $IC_{50}$ value in between **9** and **10** (Table 1).

We then measured substrate activity of the BCNA derivative for VZV TK at different compound concentrations by determining both $K_m$ and $V_{max}$ values for each compound (Table 2, SI).

Compared with the parent compound **1** (Cf 1743), the three fluoro derivatives were endowed with $K_m$ values that were somewhat (up to threefold for compound **10**) lower than for compound **1**. Whereas the $V_{max}$ value for **9** was ~2.5-fold higher and for **11** was 1.5-fold lower than noticed for **1**, the $V_{max}$ for the α-fluoro derivative **10** was markedly lower than observed for the parent compound (6–7-fold). As a result, the phosphorylating capacity ($V_{max}/K_m$) of the enzyme proved highest for the β-fluoro derivative **9** and lowest for the α-fluoro derivative **10** (Table 2).




**Scheme 1.** Reagents and conditions: (i) HBr in acetic acid, DCM, rt, 22 h; (ii) hexamethyldisilazane, ammonium sulfate, acetonitrile, 70 °C, 5 h; (iii) NaI, DCM, acetonitrile, rt, 1 week; (iv) MeONa, MeOH, 1 h, rt; (v) 4-*n*-pentylphenylacetylene, tetrakis (triphenylphosphine)Pd(0), CuI, DIPEA, DMF, rt, overnight, then CuI, TEA, 85 °C, 8 h.

**Table 2**
Kinetic values of the 2′-fluoro-substituted test compounds

| Compound | $K_m$ (μM) | $V_{max}$ (μmol/μg protein/h) | $V_{max}/K_m$ |
|---|---|---|---|
| **1** | 1.58 | 22 | 14 |
| **9** | 1.11 | 50 | 45 |
| **10** | 0.47 | 3.4 | 7.2 |
| **11** | 0.95 | 14 | 14 |

The kinetic values shown in Table 2 were derived from the Lineweaver–Burk diagrams based on the data shown in Supplementary data.

**Table 3**

| Compound | VZV $EC_{50}$[a] (μM) | | | MCC[b] (μM) |
|---|---|---|---|---|
| | OKA | YS | TK⁻ 07-1 | |
| **1** | 0.0097 | — | >20 | ⩾50 |
| **9** | 0.007 | 0.011 | >50 | >50 |
| **10** | 0.75 | — | >20 | >50 |
| **11** | 1.5 | 6.7 | >50 | ⩾50 |

[a] 50% Effective concentration, or compound concentration required to reduce viral plaque formation by 50% in the VZV-infected human embryonic (HEL) cell cultures.
[b] Minimal cytotoxic concentration, or compound concentration that results in a microscopical alteration of HEL cell morphology.

When tested in vitro against two strains of TK-competent VZV[13] (Table 3) we found that (**10**) was moderately antivirally active, being ca. 100 times less active than (**1**). The 2′,2′-difluoro BCNA (**11**) was also markedly less active than the parent drug **1**. By contrast, the β fluoro derivative (**9**) was highly active, being comparable to (**1**) in its anti-VZV activity. As previously shown,[1,2] all compounds lost activity versus VZV TK- deficient virus strains, confirming their need for VZV TK-mediated activation (phosphorylation).

As noticed before for other BCNA derivatives,[11] phosphorylation by VZV TK proved necessary, but not sufficient to display potent antiviral efficacy. In fact, the parent compound **1** showed an equal capacity for phosphorylation to that of compound **11** (difluoro-derivative) but proved ⩾100-fold more antivirally active than **11**. Instead, the β-fluoro derivative **9** could be ~3.5-fold better phosphorylated than **1**, but was found equally antivirally active as **1**. Thus, there is no correlation between antiviral potency and VZV TK affinity (substrate) properties indicating that other factors are important for eventual antiviral action.[11]

Thus, in conclusion, we report the synthesis of the α- and β-mono-2′-fluoro analogues **10** and **9** of the potent anti-VZV BCNA (**1**) and also the 2′,2′-difluoro BCNA **11**. Only the 2′-β-fluoro analogue retains full low-nanomolar potency versus VZV in cell culture.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.09.116.

## References and notes

1. McGuigan, C.; Yarnold, C. J.; Jones, G.; Velázquez, S.; Barucki, H.; Brancale, A.; Andrei, G.; Snoeck, R.; De Clercq, E.; Balzarini, J. *J. Med. Chem.* **1999**, *42*, 4479.
2. McGuigan, C.; Barucki, H.; Blewett, S.; Carangio, A.; Erichsen, J. Y.; Andrei, G.; Snoeck, R.; De Clercq, E.; Balzarini, J. *J. Med. Chem.* **2000**, *43*, 4993.
3. Hutchins, J.; Chamberlain, S.; Chang, C.; Ganguly, B.; Gorovits, E.; Hall, A.; Henson, G.; Kolykhalov, A.; Liu, Y.; Muhammad, J.; Perrone, P.; Gilles A.; Holl, S.; Madela, K.; McGuigan, C.; Patti, J. 22nd International Conference on Antiviral Research, Miami, *Antiviral Res.* **2009**, *82*, A81.
4. McGuigan, C.; Balzarini, J. *Antiviral Res.* **2006**, *71*, 149.
5. Migliore, M. D.; Zonta, N.; McGuigan, C.; Henson, G.; Andrei, G.; Snoeck, R.; Balzarini, J. *J. Med. Chem.* **2007**, *50*, 6485.
6. McGuigan, C.; Migliore, M.; Henson, G.; Patti, J.; Andrei, G.; Snoeck, R.; Balzarini, J. 21st International Conference on Antiviral Research, Montreal, *Antiviral Res.* **2008**, *78*, A1–A76, 33.
7. Parker, W. B. *Chem. Rev.* **2009**, *109*, 2880.
8. Stuyver, L. J.; McBrayer, T. R.; Thranish, P. M.; Clark, J.; Hollecker, L.; Lostia, S.; Nachman, T.; Grier, J.; Bennett, M. A.; Xie, M.-Y.; Schinazi, R. F.; Morrey, J. D.; Julander, J. L.; Furman, P. A.; Otto, M. J. *Antiviral Chem. Chemother.* **2006**, *17*, 79.
9. Tann, C. H.; Brodfuehrer, P. R.; Brundidge, S. P.; Sapino, C., Jr.; Howell, H. G. *J. Org. Chem.* **1985**, *50*, 3644.
10. *Procedure for the preparation of* (**9**). Synthesis of 5-iodo-2′-β-fluoro-2′deoxyuridine (**8**). To a stirring solution of (**7**) (2.40 g, 4.14 mmol) in anhydrous methanol (60 mL) was added NaOMe (0.49 g, 9.70 mmol) and the reaction mixture was stirred at room temperature for 1 h. After this period, the reaction was neutralized with Amberlite, filtered and concentrated to give the desired product, which was used in the following step without further purification. Synthesis of 3-(2′-β-fluoro-2′-deoxy-β-D-ribofuranosyl)-6-(4-*n*-pentylphenyl)-2,3-dihydrofuro [2,3-*d*]pyrimidin-2-one (**9**). To a solution of (**8**) (1.56 g, 4.14 mmol) in anhydrous DMF (20 mL) were added: 4-*n*-pentylphenylacetylene (2.40 mL, 12.41 mmol), tetrakis triphenylphosphine palladium(0) (0.48 g, 0.41 mmol), copper(I) iodide (0.16 g, 0.83 mmol) and DIPEA (1.44 mL, 8.27 mmol) and the reaction mixture was stirred at room temperature, under an Argon atmosphere overnight. After this period were added copper(I) iodide (0.16 g, 0.83 mmol) and anhydrous TEA (20 mL) and the reaction mixture was stirred at 85 °C for 8 h. The solvent was then removed in vacuo and the residue was triturated with DCM and stirred at room temperature for 2 h. The solid was filtered and washed with DCM to give the desired compound as light brown solid (0.78 g, 45%). A sample of this compound was further purified by filtration trough silica gel for testing. $^{19}F$ NMR (DMSO-$d_6$, 471 MHz): $\delta$ −197.85. $^1H$ NMR (DMSO-$d_6$, 500 MHz): $\delta$ 8.73 (1H, s, H-4), 7.74 (2H, d, $J$ = 8.25 Hz, Ph), 7.33 (2H, d, 8.25 Hz, Ph), 7.22 (1H, s, H-5), 6.25, 6.22 (1H, 2d, $J$ = 3.70 Hz, $J_{H-F}$ = 17.10 Hz, H-1′), 6.07 (1H, d, $J$ = 4.60 Hz, 3′-OH), 5.28 (1H, t, $J$ = 5.80 Hz, 5′-OH), 5.24, 5.14 (1H, 2dd, $J$ = 2.35 Hz, $J$ = 3.65, $J_{H-F}$ = 52 Hz, H-2′), 4.29, 4.26 (1H, 2dd, $J$ = 4.30 Hz, $J$ = 6.10, $J_{H-F}$ = 18.16 Hz, H-3′), 3.99 (1H, q, H-4′), 3.71–3.63 (2H, m, H-5′), 2.62 (2H, t, α-$CH_2$), 1.59 (2H, qn, β-$CH_2$), 1.35–1.24 (4H, m, 2 × $CH_2$), 0.86 (3H, t, $CH_3$). $^{13}C$ NMR (DMSO-$d_6$, 126 MHz): $\delta$ 13.85 ($CH_3$), 21.87 ($CH_2$), 30.32 (β-$CH_2$), 30.78 ($CH_2$), 34.87 (α-$CH_2$), 60.13 (C-5′), 73.08 (d, $J_{C-F}$ = 24.49 Hz, C-3′), 85.22 (C-4′), 86.15 (d, $J_{C-F}$ = 16.64 Hz, C-1′), 94.66 (d, $J_{C-F}$ = 191.53 Hz, C-2′) 98.60 (C-5), 107.14 (C-4a), 124.61 (Ph), 125.70 (*ipso*-C), 128.99 (Ph), 138.70 (C-4), 144.20 (*para*-C), 153.47 (C-6), 154.20 (C-2), 171.30 (C-7a). EI MS = 416.1749 ($M^+$). HPLC = $H_2O/CH_3CN$ from 100/0 to 0/100 in 30 min = retention time 23.81 min. 3-(2′-α-Fluoro-2′-deoxy-β-D-ribofuranosyl)-6-(4-*n*-pentylphenyl)-2,3-dihydrofuro [2,3-*d*]pyrimidin-2-one (**10**). $^{19}F$ NMR (DMSO-$d_6$, 471 MHz): $\delta$ −201.19. $^1H$ NMR (DMSO-$d_6$, 500 MHz): $\delta$ 8.94 (1H, s, H-4), 7.75 (2H, $H_a$) −7.32 (2H, $H_b$) ($^3J$ = 8.10), 7.22 (1H, s, H-5), 6.02 (1H, d, $J$ = 17.0, H-1′), 5.60 (1H, d, $J$ = 6.60, 3′-OH), 5.42 (1H, t, $J$ = 4.90, 5′-OH), 5.00 (1H, dd, $J$ = 3.70, $J_F$ = 52.65, H-2′), 4.26–4.20 (1H, m, H-3′), 3.95 (1H, dd, $J$ = 12.4, 2.9, H-4′), 3.77–3.67 (2H, m, H-5′), 2.61 (2H, t, $J$ = 7.6, α-$CH_2$), 1.65–1.61 (2H, m, b-$CH_2$), 1.42–1.18 (4H, m, g/d-$CH_2$), 0.86 (3H, t, $J$ = 6.90, $CH_3$). $^{13}C$ NMR (DMSO-$d_6$, 126 MHz): $\delta$ 13.84 ($CH_3$), 21.88, 30.33, 30.79, 34.88 ($C_4H_8$), 58.42 (C-5′), 66.31 (d, $J_{C-F}$ = 16.3, C-3′), 83.04 (C-4′), 89.54 (d, $J_{C-F}$ = 34.0, C-1′), 94.16 (d, $J_{C-F}$ = 185.68, C-2′), 98.56 (C-5), 107.19 (C-4a), 124.58 (C-$H_b$), 125.77 (*ipso*-C), 128.96 (C-$H_a$), 137.77 (C-4), 144.14 (*para*-C), 153.69 (C-6), 154.12 (C-2), 171.27 (C-7a). EI MS = 416.1738 ($M^+$). Anal. Calcd for $C_{22}H_{25}FN_2O_5 \cdot 0.5H_2O$: C, 62.11; H, 6.16; N, 6.58. Found: C, 61.73; H, 6.15; N, 6.41. 3-(2′-Difluoro-2′-deoxy-β-D-ribofuranosyl)-6-(4-*n*-pentylphenyl)-2,3-dihydrofuro [2,3-*d*]pyrimidin-2-one (**11**). $^{19}F$ NMR (DMSO-$d_6$, 471 MHz): $\delta$ −116.84. $^1H$ NMR (DMSO-$d_6$, 500 MHz): $\delta$ 8.75 (1H, s, H-4), 7.76 (2H, $H_a$) −7.34 (2H, $H_b$) ($^3J$ = 8.20), 7.23 (1H, s, H-5), 6.35 (1H, d, $J$ = 6.50, H-1′), 6.33–6.30 (1H, m, 3′-OH), 5.43 (1H, t, $J$ = 5.30, 5′-OH), 4.36–4.18 (1H, m, H-3′), 3.99–3.95 (1H, m, H-4′), 3.91–3.85 (1H, m, H-5′), 3.75–3.69 (1H, m, H-5′), 2.63 (2H, t, $J$ = 7.6 α-$CH_2$), 1.68–1.52 (2H, m, β-$CH_2$), 1.40–1.21 (4H, m, g/d-$CH_2$), 0.87 (3H, t, $J$ = 7.00, $CH_3$). $^{13}C$ NMR (DMSO-$d_6$, 126 MHz): $\delta$ 13.85 ($CH_3$), 21.88, 30.32, 30.79, 34.90 ($C_4H_8$), 58.62 (C-5′), 68.14 (t, $J_{C-F}$ = 22.40, C-3′), 81.11 (C-4′), 85.14 (t, $J_{C-F}$ = 31.2, C-1′), 98.37 (C-5), 107.94 (C-4a), 120.84, 122.91 (C-2′), 124.75 (C-$H_b$), 125.56 (*ipso*-C), 129.01 (C-$H_a$), 137.26 (C-4), 144.44 (*para*-C), 153.68 (C-6), 154.87 (C-2), 171.53 (C-7a). EI MS = 435.1731 (M+H). Anal. Calcd for $C_{22}H_{24}F_2N_2O_5 \cdot 0.5H_2O$: C, 59.59; H, 5.68; N, 6.32. Found: C, 59.38; H, 5.59; N, 6.25.
11. Sienaert, R.; Andrei, G.; Snoeck, R.; De Clercq, E.; McGuigan, C.; Balzarini, J. *Biochem. Biophys. Res. Commun.* **2004**, *315*, 877.
12. Procedure of the VZV thymidine kinase experiments. The $IC_{50}$ of the test compounds against phosphorylation of [$CH_3$-$^3H$] 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, pH8.0, 2.5 mM $MgCl_2$, 10 mM dithiothreitol, 2.5 mM ATP, 10 mM NaF, 1.0 mg/mL bovine serum albumin, 1 μM [$CH_3$-$^3H$] dThd (0.1 μCi), an appropriate amount of test compound and 5 μL milli Q water. The reaction was started by the addition of

enzyme, and 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 for three times 5 min in 1 mM $HCOONH_4$ while shaking, followed by 5 min in ethanol (70%). Finally, the filters were dried and assayed for radioactivity in a toluene-based scintillant. The $IC_{50}$ was defined as the drug concentration required to inhibit 1 μM thymidine phosphorylation by 50%. Thymidine kinase assays to evaluate the test compounds as a substrate for the enzyme were performed as follows: the standard reaction mixture contained 50 mM Tris HCl pH 8, 2.5 mM $MgCl_2$, 10 mM dithiothreitol, 2.5 mM ATP, 10 mM NaF, 10 μL milli Q water, test compound at various concentrations (0.5, 2, 5 and 12.5 μM) dissolved in DMSO and 5 μL of an appropriate amount (1.5 pg) of protein VZV TK in a total reaction mixture of 50 μL. The reaction mixture was incubated at 37 °C for 60 min, and the reaction was terminated by transferring the contents into 150 μL ice-cold methanol, followed after 10 min by centrifugation at 12,000*g*. The resulting samples were injected on a Waters HPLC to separate and quantify the nucleoside and 5′-monophosphates of the BCNAs. HPLC analysis to separate and quantitate the lipophilic reaction products was done on a Merck (Darmstadt, Germany) LiChroCART 125-4 RP column (5 μm) using the following gradient (flow 1 mL/min); 2 min at 98% $NaH_2PO_4$ (Acros, New Yersey, USA) 50 mM + heptanesulfonic acid 5 mM pH 3.2 (buffer) (Sigma, St. Louis, MO) and 2% acetonitrile (ACN) (Biosolve, Valkenswaard, The Netherlands); 6 min linear gradient to 80% buffer and 20% ACN; 2 min linear gradient to 75% buffer and 25% ACN; 10 min linear gradient to 65% buffer and 35% ACN; 10 min linear gradient to 50% buffer and 50% ACN; 10 min isocratic flow; 5 min linear gradient to 98% buffer and 2% ACN; 5 min equilibration at the same conditions. Metabolites of the BCNAs were determined by fluorescence detection (excitation at 340 nm and emission at 415 nm). Retention times of BCNA nucleoside and 5′-monophosphate derivatives were as follows: **1** (Cf 1743): 30.2 and 22.0 min; **9** (Cf 2852): 31.8 and 24.0 min; **10** (Cf 2792): 31.3 and 22.7 min; **11** (Cf 2819): 32.8 and 24.5 min, respectively.

13. Procedure of the anti-VZV experiments in HEL cell cultures. The laboratory wild-type VZV strain OKA and the thymidine kinase-deficient VZV strain 07/1 were used. The OKA strain was supplied by Dr. M. Takahashi, Osaka University, Osaka, Japan. The YS strain was isolated from vesicular fluid of a patient with varicella and the TK-deficient 07/1 strain was isolated alter exposure of BVaraU to VZV (YS)—infected cell cultures (Sakuma, Antimicrob. Agents Chemother. 1984, 25, 742). Confluent HEL cell cultures grown in 96-well microtiter plates were inoculated with VZV at an input of 20 PFU per well. After a 2-h incubation period, residual virus was removed and varying concentrations of the test compounds were added (in duplicate). Antiviral activity was expressed as the 50%-effective concentration required to reduce viral plaque formation after 5 days by 50% as compared with untreated controls. Cytotoxicity was expressed as the minimum cytotoxic concentration (MCC) or the compound concentration that causes a microscopically detectable alteration of cell morphology.