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Bicyclic nucleoside inhibitors of Varicella–Zoster virus: The effect of branching in the *p*-alkylphenyl side chain

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Abstract—Further to the discovery of bicyclic furanopyrimidine nucleoside analogues (BCNAs) as potent anti-VZV agents, a branched series of this family of compounds was synthesised. The aim was to study the impact of the geometry and steric hindrance in the side chain as well as lipophilic role of this moiety on biological activity. The results showed a detrimental effect of branching on antiviral activity, with a different magnitude depending on the position of branching in the side chain. This study again showed the importance of this moiety for biological activity, as well as the limited efficacy of the C log *P* value as a tool for predicting the potency of BCNAs, while suggesting an alternative rationale behind the design of future series.

We have already reported the discovery of an entirely new class of potent antiviral deoxynucleoside analogues bearing an unusual bicyclic base.[1] These bicyclic furanopyrimidines have revealed to be potent and selective inhibitors of Varicella–Zoster virus replication and several efforts have been made in our group to investigate the structural requirements needed for optimal antiviral activity.

Modifications of the side chain, the base and the sugar moiety have been considered.[2] In particular, previous SAR studies have revealed as an absolute requirement for antiviral activity—a long lipophilic alkyl or alkylphenyl side chain; in fact, modifications involving a significant increase of the polarity in the side chain decreased the activity of the corresponding analogues. As a result, the potency of these nucleosides seems to be strictly related to their C log *P* values, with an optimal value falling between 2.5 and 3.5.

Among all the derivatives synthesised, the most active series reported so far is the one bearing a phenyl group in the side chain (Fig. 1). In particular the *p*-pentylphenyl derivative (**4**) displays an EC_{50} below 1 nM and a selectivity index value of ca. 1,000,000.[3] It is currently under development for use in shingles by FermaVir Pharmaceuticals.

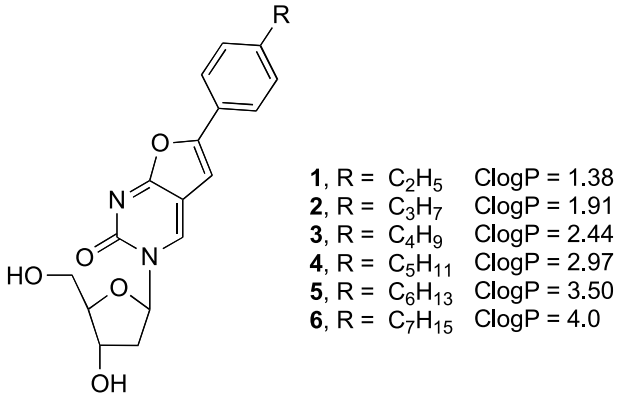

Figure 1. Bicyclic furanopyrimidines (BCNAs) belonging to the most active series and their corresponding C log *P* values.

To understand the role of the alkyl group in biological activity, we here report the synthesis and biological evaluation of a new class of BCNA derivatives characterised by a branched side chain (Fig. 2). This represents the first example of branched *p*-alkylphenyl BCNAs.

These modifications would be of interest for investigating the effect of steric hindrance and conformational restriction and also of the, previously predictive, C log *P*

Keywords: VZV; BCNA; Nucleoside analogues; C log *P*; Shingles.
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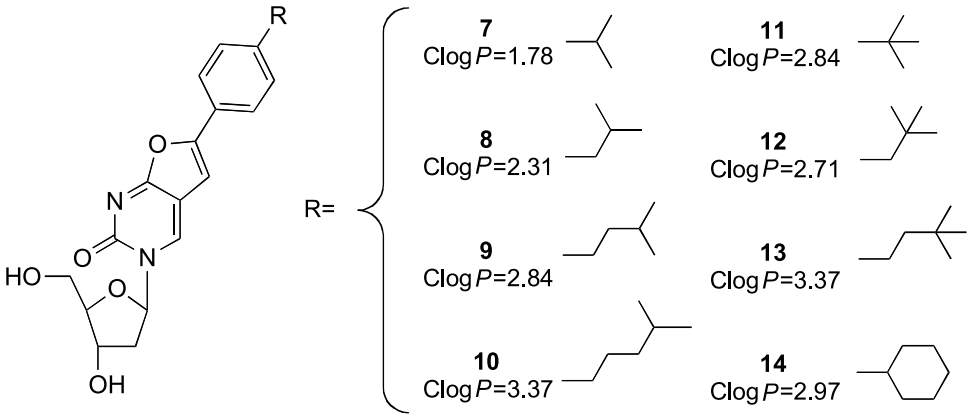


Figure 2. Target derivatives and their corresponding Clog*P* value.

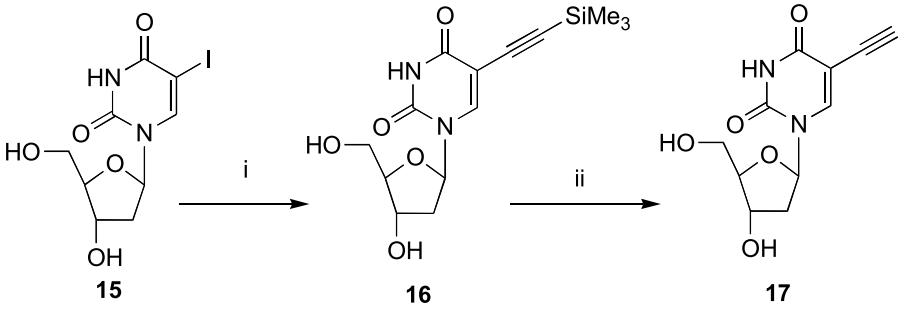


Figure 3. Synthesis of EDU (**17**). Reagents and conditions: (i) HC≡CSiMe$_3$, DIPEA, Pd(Ph$_3$P)$_4$, CuI, DMF, rt, overnight, 81%; (ii) NH$_4$OH, MeOH, rt, overnight, 75%.

value. Compound **14** was particularly indicative for our purposes, having the same Clog*P* value as the most active *p*-pentylphenyl BCNA (**4**).

The general synthetic pathway for the synthesis of bicyclic furanopyrimidines (BCNAs) involves a Pd-catalysed coupling between 5-iodo-2′-deoxyuridine (IDU, **15**) and the appropriate aryl acetylenes.[1] Unfortunately, the aryl acetylenes required for the synthesis of these new analogues were not commercially available.

Therefore, we considered the building of the alkynyl moiety on the nucleoside, leading to 5-ethynyl-2′-deoxyuridine (**17**) as the synthon for subsequent coupling with appropriate aryl iodides (Fig. 3).

Thus, the desired aryl iodides were synthesised via an aromatic iodination of the appropriate alkylbenzene in the presence of 2 equiv of I_2 and 2 equiv of $AgNO_2$,[4] which led to compounds in good yield as a mixture of ortho and para regioisomers, the ratio of which was determined by ^{1}H NMR (Fig. 4).

Due to the fact that it was not possible to separate the regioisomers by flash chromatography, the aryl iodides were used as mixtures for the following synthetic steps.

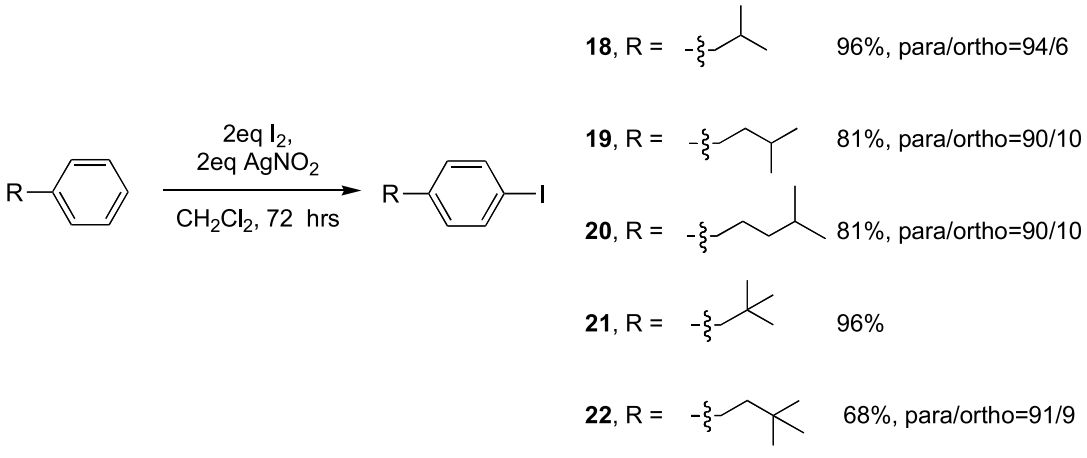


Figure 4. Synthesis of the required aryl iodides (**18**–**22**), corresponding yields and para/ortho ratios.

The alkylphenyl starting materials necessary for the synthesis of compounds **20** and **22** were not commercially available and they had to be synthesised.

As reported in the literature by Wei and Taylor,[5] styrene faces attack by a range of organolithium reagents and subsequent treatment of the intermediate anion with MeOH gives access to several alkylbenzene analogues. This approach was chosen for the synthesis of the desired (3,3-dimethyl)butylbenzene (**25**) (Fig. 5).

The same synthetic pathway was chosen for the preparation of (4-methyl)pentylbenzene (**28**). As shown in Figure 6 the required *iso*-propyl lithium **29** was prepared by reacting *iso*-propyl iodide with excess *tert*-BuLi in diethyl ether at −78 °C. Such an exchange is indicated to be irreversible in the presence of 2 equiv of *tert*-BuLi.[6] After generation of the desired primary alkyl lithium derivative, the residual *tert*-BuLi was eventually eliminated by proton abstraction from diethyl ether, by simply allowing the reaction to stand at room temperature for ca. 1 h. The reaction mixture then reacted with styrene to afford **28**, following the previously described procedure.

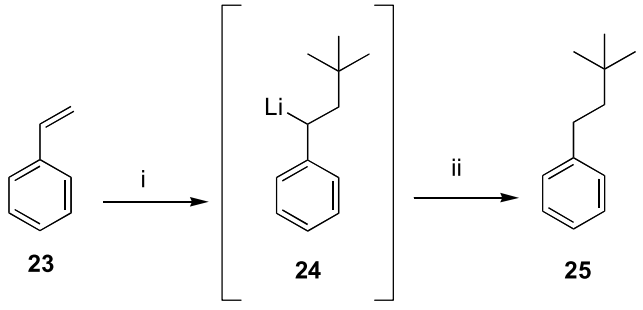


Figure 5. Synthesis of (3,3-dimethyl)butylbenzene (**25**). Reagents and conditions: (i) *tert*-BuLi, Et_2O, −78 °C, 30 min; (ii) MeOH, −78 °C, 30 min, 86%.

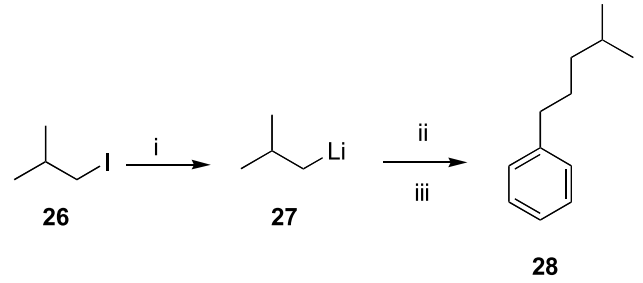


Figure 6. Synthesis of 4-methylpentylbenzene (**28**). Reagents and conditions: (i) *tert*-BuLi, Et_2O, −78 °C, 10 min, rt, 1 h; (ii) styrene (**23**), Et_2O, −25 °C, 30 min; iii) MeOH, −25 °C, rt, 30 min, 57%.

The product **28** was successfully isolated and NMR analysis excluded the presence of any side product, which may have resulted from the attack of any residual *tert*-BuLi on styrene.

Converse to the iodination with molecular iodine, a different synthetic pathway was preferred for the last outstanding *p*-iodocyclohexylbenzene (**30**), and this was achieved in 57% yield, starting from cyclohexylaniline via formation of the corresponding diazonium salt (Fig. 7).[7]

Once all the necessary *p*-alkylaryliodides were successfully synthesised, they were subsequently coupled with EDU (**17**) in the presence of $Pd(Ph_3P)_4$, CuI and triethylamine to achieve the corresponding 5-arylethynyl-2′-deoxyuridines (**31**–**38**). A copper-catalysed cyclisation then led to the formation of bicyclic furanopyrimidine ring derivatives (**7**–**14**) (Fig. 8).[8]

While 5-arylethynyl-2′-deoxyuridines **31** and **38** were isolated and characterised, the two synthetic steps were carried out in a one-pot procedure for the synthesis of other cyclised compounds in the series, and yields displayed in Figure 8 refer to EDU as the starting material.

The key spectroscopic evidence for the cyclisation is the disappearance of the NH peak in the ^1H NMR (ca. 11.6 ppm) and the presence of a H-5 peak in the region 7.10–7.30 ppm, depending on the aryl substituent.

Compared to the yields achieved for **7** and **11** (respectively, 63% and 52% as overall yields from EDU), we observed a decrease in the efficiency for the one-pot procedure. Besides the inevitable loss of material due to a more difficult purification, these lower yields could be due to the formation of 5,6-disubstituted analogues as side products.

As shown in Figure 9, during the synthesis of **13**, the corresponding 5,6-disubstituted analogue **39** was, in fact, isolated and characterised. Analogous fluorescent compounds at similar R_fs were detected by TLC in all the other reaction mixtures carried out in one pot.

These disubstituted derivatives are believed to be the result of a side reaction occurring during the one-pot cyclisation. Indeed, the suggested mechanism for their formation is predicted to involve the reactivity of the 5-arylethynyl nucleoside intermediates, the triple bond of which would react with the Pd-complex formed with the remaining excess of aryl iodide. Given this, the isolation of the 5-arylethynyl intermediates would prevent the formation of these side products, as supported by the higher yields obtained for **7** and **11**.

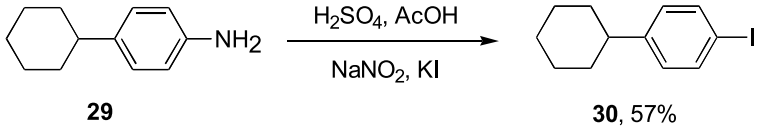
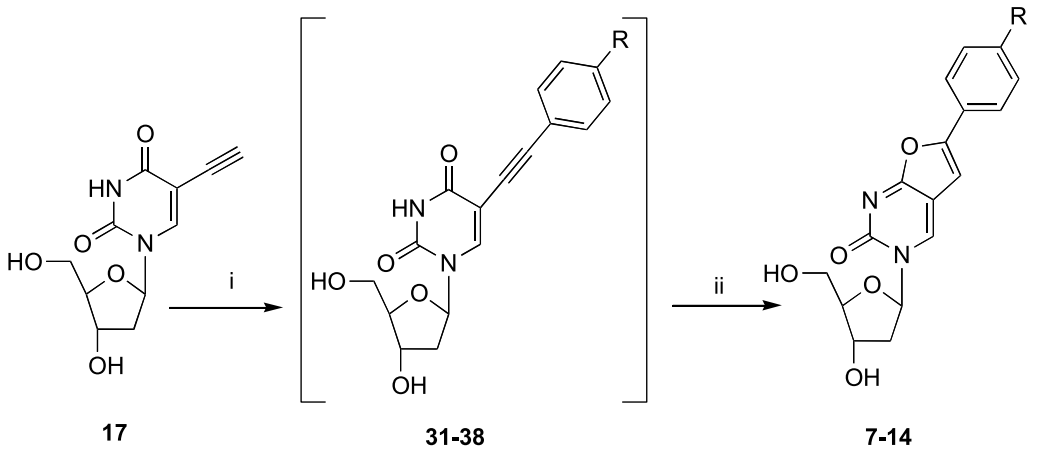


Figure 7. Synthesis of *p*-iodocyclohexylbenzene (**30**).



R	Cpd	yield	Cpd	yield
$CH(CH_3)_2$	**31**	85%	**7**	63%
$CH_2CH(CH_3)_2$	**32**	-	**8**	20%
$CH_2CH_2CH(CH_3)_2$	**33**	-	**9**	19%
$CH_2CH_2CH_2CH(CH_3)_2$	**34**	-	**10**	20%
$C(CH_3)_3$	**35**	63%	**11**	52%
$CH_2C(CH_3)_3$	**36**	-	**12**	30%
$CH_2CH_2C(CH_3)_3$	**37**	-	**13**	26%
C_6H_{11}	**38**	-	**14**	41%

Figure 8. Synthesis of branched derivatives (**7**–**14**). Reagents and conditions: (i) ArI, Et_3N, $Pd(Ph_3P)_4$, CuI, DMF, rt, overnight; (ii) MeOH, CuI, reflux, 4 h.

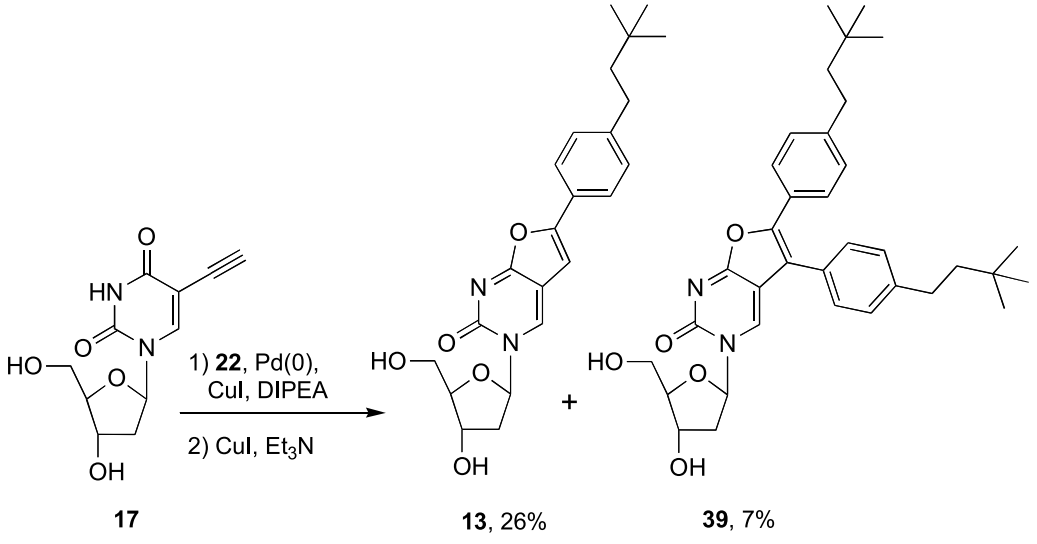


Figure 9. Synthesis of the target structure **13**. The 5,6-disubstituted analogue **39** was also isolated.

Furthermore, the fact that the synthetic aryl iodides **18**–**20** and **22** were used as a mixture of ortho and para regioisomers for subsequent coupling with EDU could also have contributed to the low yields in the coupling reaction. Although we failed to isolate any ortho bicyclic furanopyrimidine analogue, the possibility that the 5-(*o*-alkylaryl)ethynyl derivatives had been formed cannot be ruled out. As a matter of fact, a parallel ongoing work has found that the cyclisation step of some ortho-substituted 5-(alkylaryl)ethynyl analogues appears to require more severe conditions to achieve good yields, while no significant difficulties had been encountered at the stage of coupling. On account of this, the amount of the cyclised ortho isomer may not have been considerable. Yet, taking into account the relatively poor yields of the whole process for **8**–**10** and **13**, it seems likely that these conditions could have affected, to some extent, the yields of the coupling reactions. All of the spectroscopic

Table 1. Antiviral activities for compounds **7**–**14**; samples were dissolved in neat DMSO and diluted with biological media to generate ca. 50–200 μM stock solutions containing 1% DMSO

Compound	R	Clog P[b]	EC_{50}[a] (μM)				MCC[d] (μM)	CC_{50} (μM)[e]
			VZV OKA	VZV YS	TK^-VZV 07/1[c]	TK^-VZV YS/R[c]		
7	$CH(CH_3)_2$	1.78	6.9	7.9	>20	>50	⩾50	45
8	$CH_2CH(CH_3)_2$	2.31	0.048	0.051	>200	>2	>200	>200
9	$C_2H_4CH(CH_3)_2$	2.84	0.0017	0.0011	>50	>2	⩾50	>200
10	$C_3H_6CH(CH_3)_2$	3.37	>80	ND[f]	>80	ND[f]	400	>200
11	$C(CH_3)_3$	2.18	>50	>20	>50	>50	⩾50	>200
12	$CH_2C(CH_3)_3$	2.71	>80	ND[f]	>80	ND[f]	400	140
13	$C_2H_4C(CH_3)_3$	3.37	0.33	0.31	⩾80	ND[f]	⩾80	>200
14	C_6H_{11}	2.97	1.4	3.1	>20	>20	>20	>200
ACV	—	—	1.5	3.1	40	53	>200	>400
1	C_2H_5	1.38	0.09	0.07	>50	>20	200	123
2	C_3H_7	1.91	0.01	0.008	>20	>20	⩾50	188
3	C_4H_9	2.44	0.0022	0.0005	>5	>5	⩾20	>200
4	C_5H_{11}	2.97	0.0003	0.0001	>20	>5	⩾20	>200
5	C_6H_{13}	3.50	0.0005	0.0001	>5	>5	20	18
6	C_7H_{15}	4.0	0.0054	0.003	>5	>5	5	18
40	Ph	2.24	0.031	0.032	>5	>5	>200	>200

[a] EC_{50}, effective concentration (μM), required to reduce virus plaque formation by 50%.
[b] Values calculated using Clog P version 1.0.0. Biobyte, P.O. Box 517, Claremont, CA 91711, USA.
[c] TK^-, thymidine kinase deficient.
[d] MCC, minimal cytotoxic concentration (μM), required to alter microscopically detectable cell morphology.
[e] CC_{50}, 50% cytotoxic concentration, required to inhibit Hel cell growth by 50%.
[f] ND, not determined.

data confirm the isolated products **7**–**14** to be pure para isomers.

The synthesised compounds **7**–**14** were evaluated for their ability to inhibit the replication of VZV in cell culture. Table 1 contains data relating to two strains of thymidine kinase-competent (TK^+) VZV and also two strains of thymidine kinase-deficient (TK^-) VZV, with data also given for aciclovir (ACV) as a reference compound.

Data corresponding to the *p*-alkylphenyl series (**1**–**6**) are also included in the table, as well as the results related to compound **40**, which has a biphenyl group as the side chain. Cytotoxicity data are given for each compound in two assays.

In general, all the compounds show an activity lower than those of the potent *n*-alkylphenyl BCNA homologues, indicating a negative effect of the branching. This is particularly evident in the *tert* series, where the only active derivative is **13**, while **11** and **12** did not show any activity at the highest subtoxic concentrations tested (50 and 80 μM, respectively). For the *iso* and *tert* series, there seems to be a tendency for an increase of activity with the increase of the chain length. Indeed, in the *iso* series, the potency increases by ca. 140-fold from **7** to **8** and by ca. 30-fold from **8** to **9**. Similarly, in the *tert* series, while **11** and **12** did not show any significant activity, **13** is endowed with an antiviral potency higher than that of aciclovir. In this context, the loss of activity of the *iso*-hexyl derivative (**10**) is particularly surprising and it indicates that the branching at the C-5 position on the alkylphenyl side chain is not tolerated. The fact that the branching could have a different influence depending on its position in the alkylphenyl side chain can be supported further by a large difference in activity between the *iso*-propyl derivative **7** and *n*-propylphenyl BCNA **2** (ca. 700-fold). This suggests a detrimental effect of branching at the position α to the phenyl group, supported further by the inactivity of **11**.

When we compare the results for **10** and **13** to that of *n*-hexylphenyl BCNA (**5**), we observe a striking difference in activity between compounds that have comparable Clog P values. On this basis, the result for the cyclohexyl derivative **14** is particularly interesting. Indeed, although **14** and the extremely potent *n*-pentylphenyl (**4**) derivatives have the same Clog P value, they show a difference in activity of 2800-fold. It is also of interest to consider the result for the biphenyl derivative **40**, which displays an antiviral activity that remains between that of **4** and **14**, yet we find that the Clog P values of the compounds are only slightly different.

Although these losses of activity could be explained by difference in the geometry and steric hindrance of the side chain between the derivatives, the reliability of comparison between the Clog P values of bicyclic nucleoside analogues with different structures could also be questioned.

To assess the possibility to use the Clog P value for predicting the optimal antiviral activity of BCNAs for different series, it was decided to carryout an experimental measurement of their log P values, choosing the *n*-pentylphenyl **4** as the reference.

To carry out the assay, equal amounts of water and *n*-octanol (99+%) were mixed overnight to attain a mutual saturation. After separation, an appropriate amount of **4** was dissolved in *n*-octanol at a concentration lower than 1 mM. However, when the water was added and the two phases were mixed by shaking for 6 h,

UV spectroscopy did not show any significant difference in absorbance from the octanol solution before and after the extraction with water, indicating that no detectable amount of compound was transferred through the aqueous phase.

An aliquot of the aqueous layer was also analysed, but no UV spectrum could be detected.

In view of these results, 1 mg of **4** was first suspended in 100 ml water, and left stirring for 6 h, and occasionally sonicated. The suspension was filtered giving a clear solution. However, when an aliquot of this aqueous solution was analysed, no significant absorbance could be detected and the UV spectrum could not be registered. Therefore, due to the pronounced lipophilicity of the compound, the experimental measurement of the log*P* value of **4** could not be carried out using this method and the available software for the calculation of log*P* remains the most convenient tool to estimate the log*P* value of such derivatives.

In conclusion, the branching of the alkylaryl side chain of BCNAs leads to a decrease in their biological activity against VZV, with a different impact depending on the position of the branching in the chain. In view of these results, it is clear that Clog*P* is not the only factor influencing the biological activity, the geometry and steric hindrance in the alkylaryl side chain are also important factors to be considered in predicting the antiviral activity of BCNAs.

Acknowledgments

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8. *General experimental procedure for the one-pot coupling and cyclisation given for compound (**8**)*. To a stirred solution of **17** (450 mg, 1.77 mmol) in anhydrous dimethylformamide (10 ml), at room temperature and under a nitrogen atmosphere, triethylamine (0.27 ml, 1.95 mmol), **18** (554.5 mg, 2.13 mmol), tetrakis(triphenylphosphine)palladium(0) (102.6 mg, 0.089 mmol) and copper(I) iodide (33.8 mg, 0.18 mmol) were added. The reaction mixture was stirred for 15 h, after which time TLC (EtOAc) showed complete conversion of the starting material. Et_3N (5 ml) and CuI (101.4 mg) were added and the reaction mixture was then heated at 80 °C. The reaction was monitored by TLC (EtOAc/MeOH 95:5) and after 4 h the solvent was removed in high vacuo. The resulting residue was dissolved in dichloromethane/methanol (1:1), and then excess Amberlite IRA-400 (HCO_3^- form) was added and the mixture was stirred at room temperature for 30 min. The reaction mixture was then filtered and washed with methanol and the combined filtrate was evaporated to dryness. The crude residue was purified by flash chromatography (EtOAc), and the product was loaded absorbed on silica, after being dissolved in hot methanol. The appropriate fractions were combined and the solvent was removed in vacuo. A second purification was necessary ($CHCl_3$/MeOH 95:5) and the recovered product was subsequently crystallised with MeOH to yield the product as a white solid (136.5 mg, 20%). ^{1}H NMR (DMSO-d_6; 300 MHz) δ 8.85 (1H, s, H-4), 7.74 (2H, H-A)-7.29 (2H, H-B) (AB system, J = 8.1 Hz), 7.22 (1H, s, H-5), 6.19 (1H, ψt, J = 6.0 Hz, H-1′), 5.32 (1H, d, J = 4.2 Hz, 3′-OH), 5.21 (1H, t, J = 5.0 Hz, 5′-OH), 4.28–4.25 (1H, m, H-3′), 3.94–3.93 (1H, m, H-4′), 3.75–3.61 (2H, m, H-5′), 2.48–2.38 (3H, m, H-2′, α-CH_2), 2.15–2.07, (1H, m, H-2′), 1.90–1.82, (1H, m, CH), 0.87 (6H, d, J = 6.6 Hz, CH_3). ^{13}C NMR (DMSO-d_6; 75 MHz) δ 22.5 (CH_3), 29.9 (CH), 41.6, 44.7, 61.0 (C-2′, C-5′, α-CH_2), 69.9 (C-3′), 87.9, 88.5 (C-1′, C-4′), 99.1 (C-5), 107.2 (C-4a), 124.8, 130.0 (C-Ph), 126.3 (C-ipso), 138.2 (C-4), 143.2 (C-para), 154.1, 154.2 (C-6, C-2), 171.4 (C-7a).