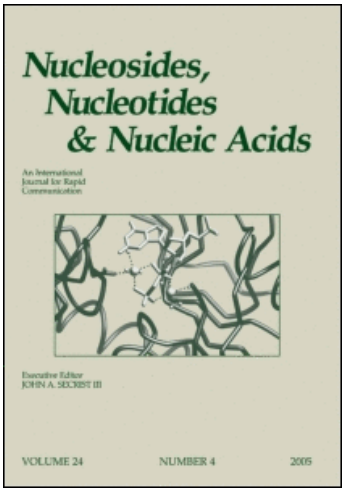

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Synthesis and Antiviral Evaluation of Some 3′-Fluoro Bicyclic Nucleoside Analogues[†]

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ABSTRACT

The synthesis of 3′-fluoro analogues of recently discovered highly potent anti-VZV furanopyrimidine deoxynucleosides (BCNAs) is herein reported, for both the alkyl and alkylphenyl series. The compounds are tested against a range of herpes viruses and display poor activity, strongly supporting the notion of the importance of the presence of a 3′-OH for antiviral activity.

Key Words: VZV; Bicyclic nucleoside analogues; 3′-OH.

INTRODUCTION

We have reported[1] the potent and selective anti-VZV (Varicella Zoster Virus) activity for bicyclic furanopyrimidine deoxynucleosides such as **1** (Figure 1). The corresponding alkylphenyl analogues **2** are even more potent, with EC_{50} values vs.

[†]In honor and celebration of the 70th birthday of Professor Leroy B. Townsend.

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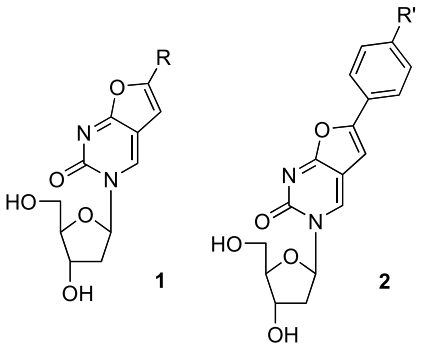
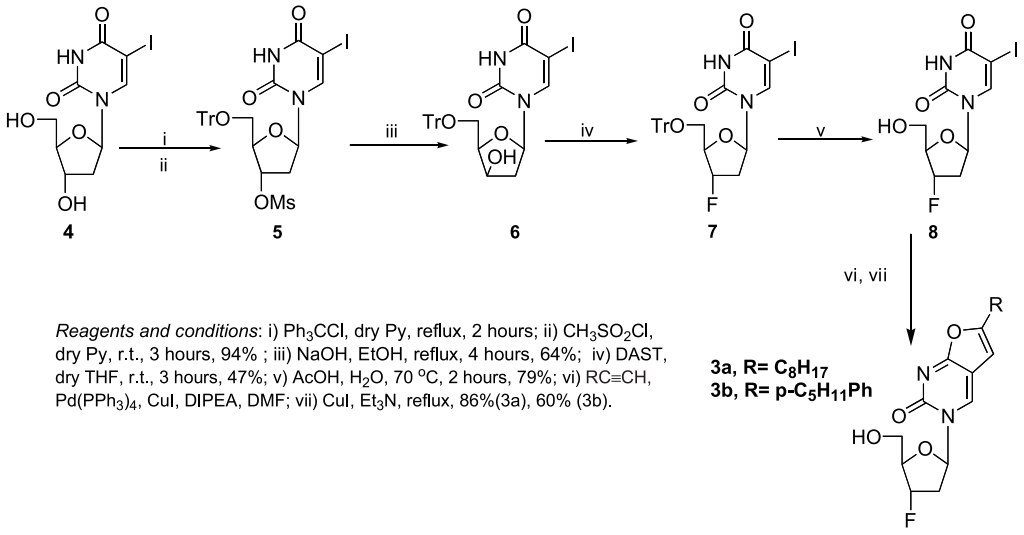


Figure 1. Structures of potent anti-VZV bicyclic furanopyrimidines (BCNAs). For optimal activity R is C_8H_{17} and R′ is C_5H_{11}.

VZV in vitro in the higher picomolar range.[2] The compounds exhibit complete specificity for VZV with no activity detectable against any other DNA or RNA viruses studied. We have now prepared a moderate series of compounds of this general family and recently reviewed the SAR[3,4] and biochemistry[5,6] of these agents.

Whilst we have carried out fairly extensive studies in the base region of these compounds, we have to date reported little variation in the sugar. We did note[4] that the 3′-methoxy analogue of (**1**), in fact with a C_{10} alkyl side chain, was > 10,000 times less active than the parent 2′-deoxy nucleoside. This indicated little tolerance for modification at the 3′-position.

The introduction of fluorine atoms in the sugar ring of nucleosides is well established both as a means of stabilising the glycosidic bond, as in FMAU[7] or indeed as to afford an antiviral effect, as in FLT.[8–10]



Reagents and conditions: i) Ph_3CCl, dry Py, reflux, 2 hours; ii) CH_3SO_2Cl, dry Py, r.t., 3 hours, 94% ; iii) NaOH, EtOH, reflux, 4 hours, 64%; iv) DAST, dry THF, r.t., 3 hours, 47%; v) AcOH, H_2O, 70 °C, 2 hours, 79%; vi) RC≡CH, $Pd(PPh_3)_4$, CuI, DIPEA, DMF; vii) CuI, Et_3N, reflux, 86%(3a), 60% (3b).

Scheme 1.

Table 1.

Compound	EC_{50}/μM				CC_{50}/μM
	VZV OKA	VZV YS	VZV TK⁻07	VZV TK⁻YS	
3a	> 20	> 20	> 20	> 200	> 200
3b	> 2	> 2	> 2	> 2	5.2
1	0.008	0.024	> 50	> 50	> 50
2	0.0003	0.0001	> 5	> 5	> 200

Thus, we sought the synthesis of analogues **3** of **1** and **2** with a 3′-fluorine substituent. The key synthon required for this preparation, following our established procedures[1,2] was 5-iodo-2′,3′-dideoxy-3′-fluorouridine. This type of compound has been prepared previously[10,11] for the synthesis of 3′-fluoro analogues of thymidine and uridine, and we closely followed these published procedures (Scheme 1).

Thus, selective 5′-protection of 5-iodo-2′-deoxyuridine (IDU, **4**) with trityl chloride followed by treatment with mesyl chloride gave **5** in 94% yield. Following the published procedure[11] the configuration of the 3′-group was readily inverted to give the xylo compound **6** in 64% using refluxing ethanic sodium hydroxide. The 3′-β configuration of **6** was confirmed by a positive NOESY experiment (data not shown).

Treatment of **6** with DAST[12] in dry THF at ambient temperature gave the 5′-trityl-3′-fluoro nucleoside **7** in moderate yield, and the 5′-trityl group was removed under standard acidic conditions to yield **8** as the key synthon. The successful introduction of a fluorine into **8** was confirmed by ^{19}F-NMR, with a signal at δ—174 which showed a large doublet splitting (J = ca. 50Hz) to the 3′-H in the ^{1}H-NMR and to the 3′-C (J = ca. 175 Hz) in the ^{13}C NMR. All of these data are consistent with literature values on close analogues.[11] The stereochemistry of the fluorine atom in **7** (and thus **8**) was fully confirmed as alpha by a NOESY experiment. Thus a clear cross peak between H-1′ and H-2′ (alpha) distinguished the two H2′ protons, with the beta proton being more upfield (ca. 2.6, 2.4 ppm). A clear cross peak is hence noted between H-2′ (beta) and the H-3′ proton, proving the orientation of the 3′-fluorine as alpha.

Following our established procedures[1,2] synthon **8** was readily converted into the octyl (**3a**) and p-pentylphenyl (**3b**) BCNAs using a two-step Pd- and Cu-catalysed process (Scheme 1).[a] Full spectroscopic, spectrometric and analytical data completely supported the structure and purity of **3a–b** and confirmed the retention of a 3′-alpha-F Substituent.

Thus, compounds **3a–b** were evaluated as inhibitors of VZV in vitro following our established procedures, and using standard compounds **1** and **2** as positive biological controls,[1,2] with data being presented in the Table 1.

As noted in the Table 1 compounds **3a–b** display no detectable anti-VZV activity at the highest concentration tested; **3a** is thus > 1000 times less active than its parent deoxynucleoside **1** and **3b** is > 10,000 times less active than **2**. Interestingly, **3b** does display some cytotoxicity, at ca. 5 μM. This is unusual for compounds of the BCNA family which are in general non-cytotoxic, although some long chain analogues of **2** did display toxicity at ca. 20 μM.[2]

[a]Selected experimental procedures and data for **3a–b**: 3-(2,3-Dideoxy-3-fluoro-β-D-ribofuranosyl)-6-(octyl)-2,3-dihydrofuro-[2,3-*d*]pyrimidin-2-one (**3a**).

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These data clearly show that the 3′-fluoro analogues of **1** and **2** are not active against VZV, and that the 3′-OH appears to be essential for antiviral activity. This confirms our earlier conclusion, based on the 3′-methyl ether[4] and implies little tolerance in general for 3′-modification. Most likely, it may be that a free 3′-OH is necessary for (VZV) thymidine kinase-mediated activation of those agents, which is thought to be pre-requisite for anti-VZV activity,[5] or that a subsequent metabolic step depends on the presence of a free 3′-OH, or both. We are currently pursuing a number of strategies to address these questions.

To a stirred solution of 5-iodo-2′,3′-dideoxy-3′-fluorouridine (200 mg, 0.562 mmol) in dry dimethylformamide (5 ml) at room temperature under a nitrogen atmosphere, 1-decyne (0.3 ml, 1.68 mmol), *tetrakis*(triphenylphosphine) palladium (0) (65 mg, 0.0562 mmol), copper (I) iodide (21 mg, 0.112 mmol) and diisopropylethylamine (0.2 ml, 1.12 mmol) were added. The reaction mixture was stirred at room temperature for 19 hours, after which time TLC (chloroform/methanol 95:5) showed complete conversion of the starting material. Copper(I) iodide (21 mg, 0.112 mmol), and triethylamine (8 ml) were added to the mixture which was subsequently refluxed for 8 hours. The reaction mixture was then concentrated in vacuo, and the resulting residue was dissolved in dichloromethane/methanol (1:1) (6 ml), and an excess of Amberlite IRA-400 (HCO_3^- form) was added and stirred at room temperature for 30 minutes. Then the resin was filtered, washed with methanol and the combined filtrate was evaporated to dryness. The crude product was purified by silica column chromatography, using an eluent of chloroform/methanol (95:5). The appropriate fractions were combined and the solvent was removed in vacuo to yield the product, which was further purified by trituration with methanol, yielding the pure product (178 mg, 86%) as a white solid. Mp: > 240°C (decomposes).

IR (KBr): 3338.0 (OH), 2921.9 (aliphatic), 1671.9 (CO amide), 1112.5 (C–F). ^{1}H-nmr (d_6-DMSO; 300 MHz): 8.57 (1H, s, H-4), 6.53 (1H, s, H-5), 6.20 (1H, dd, J = 5.6 and 8.3 Hz, H-1′), 5.36 (1H, dm, J = 49.3 Hz H-3′), 5.22 (1H, t, J = 5.2 Hz, 5′-OH), 4.33 (1H, dm, J = 26.2 Hz, H-4′), 3.64 (2H, m, H-5′), 2.74 (1H, m, H-2_a′), 2.63 (2H, t, J = 7.3 Hz, α-CH_2), 2.31–2.08 (1H, m, H-2_b′), 1.59 (2H, m, CH_2), 1.23 (10H, m, 5 × CH_2), 0.84 (3H, t, J = 6.6 Hz, ω-CH_3). ^{13}C-nmr (d_6-DMSO; 75 MHz): 14.3 (CH_3), 22.4, 26.7, 27.7, 28.7, 28.9, 29.0, 31.6 (7 × CH_2), 39.5 (d, J = 20.2 Hz, C-2′), 61.0 (d, J = 11.1 Hz, C-5′), 86.5 (d, J = 22.5 Hz, C-4′), 88.0 (C-1′), 95.2 (d, J = 174.2 Hz, C-3′), 100.1 (C-5) 107.0 (C-4a), 136.9 (C-4), 154.1 (C-6), 158.9 (C-2), 171.7 (C-7a). MS (ES^+) m/e 389 (MNa^+, 100%), 271 (baseNa^+, 10%). Accurate mass: $C_{19}H_{27}N_2O_4FNa$ requires : 389.1853; found: 389.1847.

3-(2,3-Dideoxy-3-fluoro-β-D-ribofuranosyl)-6-(4-pentylphenyl)-2,3,-dihydro-furo-[2,3-*d*]pyrimidin-2-one (3b). This was prepared entirely as outlined for (**3a**) above, but using 4-*n*-pentylphenylacetylene, to yield the product (235 mg, 60%) as a white solid. Mp: > 240°C (decomposes).

IR (KBr): 3354.9 (OH), 2921.3 (aliphatic), 1668.7 (CO amide), 1112.2 (C–F). ^{1}H-nmr (d_6-DMSO; 300 MHz): 7.99 (1H, s, H-4), 7.74 (2H, H_a)—7.33 (2H, H_b) (AA′BB′ system, J = 8.1 Hz), 7.22 (1H, s, H-5), 6.25 (1H, dd, J = 5.7 and 7.9 Hz, H-1′), 5.39 (1H, dm, J = 50.1 Hz, H-3′), 5.28 (1H, t, J = 5.1 Hz, 5′-OH), 4.38 (1H, dm, J = 25.9 Hz, H-4′), 3.70 (2H, m, H-5′), 2.79 (1H, m, H-2_a′), 2.61 (2H, t, J = 7.5 Hz, α-CH_2), 2.39–2.16 (1H, m, H-2_b′), 1.65 (2H, m, CH_2), 1.30 (4H, m, 2 × CH_2), 0.86 (3H, t, J = 6.7 Hz, CH_3). ^{13}C-nmr (d_6-DMSO; 75 MHz): 14.3 (CH_3), 22.3, 30.8, 31.2, 35.3

(4 × CH_2), 39.2 (d, J = 19.8 Hz, C-2′), 61.0 (d, J = 11.1 Hz, C-5′), 86.6 (d, J = 22.6 Hz, C-4′), 88.2 (C-1′), 95.1 (d, J = 174.3 Hz, C-3′), 99.0 (C-5) 107.5 (C-4a), 124.9 (C-H_b), 126.1, (C-*ipso*), 129.4 (C-H_a) 138.0 (C-4), 144.5 (C-*para*), 154.11 (C-6), 154.4 (C-2), 171.5 (C-7a). MS (ES^+) m/e 423 (MNa^+, 100%). Accurate mass: $C_{22}H_{25}N_2O_4FNa$ requires : 423.1696; found: 423.1700. Anal. Calcd for $C_{22}H_{25}N_2O_4F$: C, 65.99%; H, 6.29%; N, 7.00%. Found: C, 66.19%; H, 6.05%; N, 6.87%.

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