Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 22, pp. 2667-2672, 1994
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0960-894X/94 $7.00+0.00

0960-894X(94)00397-1

INHIBITORY ACTIVITIES OF HERPES SIMPLEX VIRUSES TYPE 1 AND 2 AND HUMAN CYTOMEGALOVIRUS BY STEREOISOMERS OF 2'-DEOXY-3'-OXA- 5(E)- (2-BROMOVINYL)URIDINES AND THEIR 4'-THIO ANALOGUES.

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Abstract. Two series of optically pure 2,4-disubstituted 1,3-dioxolanes and -1,3-oxathiolane nucleosides containing (E)-5-(2-bromovinyl)uracil were synthesized and assayed for activity against HSV-1, HSV-2 and HCMV replication *in vitro*. The β-L (2S,4S) dioxolane nucleoside **10** displayed significant activity against HSV-1, whereas the β-D (2R,4R) oxathiolane **14** demonstrated potent activity against HSV-2. The α-L (2S,4R) dioxolane **11** and oxathiolane **17** were moderately active against HSV-1 and HSV-2, respectively.

Heterosubstituted 2',3'-dideoxynucleoside analogues have emerged as an important class of antiviral agents.[1] For example, some 2,5-disubstituted 1,3-oxathiolanes and 2,4-disubstituted dioxolanes with pyrimidines[2-5] or purine[1,6-8] bases possess potent activities against the replication of the human immunodeficiency viruses (HIV) and hepatitis-B virus (HBV). (-)-2'-Deoxy-3'-thiacytidine (lamivudine, 3TC™) is in advanced clinical trials for AIDS and HBV infections[9] and its 5-fluoro analogue, (-)-FTC, has shown promise as a selective agent.[10] Despite the impressive selectivity of analogues towards the HIV and HBV viruses, no activity in this series has yet been reported against the herpes simplex virus (HSV) type 1 and 2 and human cytomegalovirus (HCMV).

Sometime ago (E)-5-(2-bromovinyl)-2'-deoxyuridine (brivudine, BVDU) was discovered as a potent and selective anti-herpes virus agent with clinical efficacy.[11] The remarkable specificity of BVDU as an anti-herpetic agent is probably determined, at least in part, by the presence of (E)-5-(2-bromovinyl)uracil (BVU) base. The related nucleosides 1-β-D-arabinofuranosyl-E-5-(2-bromovinyl)uracil (sorivudine, BV-araU),[12] carbocyclic BVDU,[13] 4'-thio BVDU[14] and their congeners[15] have demonstrated potent anti-herpetic activity and metabolic

stability against pyrimidine phosphorylases. All of the above BVU nucleosides feature the 3'-hydroxyl moiety which is believed to be necessary for anti-herpetic activity. Herein we describe the enantioselective synthesis and anti-herpetic properties of BVU nucleosides containing oxathiolanes and dioxolanes, which serve as surrogates of 2'-deoxyribofuranose and 2'-deoxy-4'-thioribofuranose.

Our recent syntheses of optically pure dioxolanes[5,16] and oxathiolanes[17,18] have provided a set of intermediates appropriate for the preparation of the desired nucleosides. From the outset, we envisioned that the acetate derivatives **1-4** could serve as starting material for coupling with persilylated BVU base **5**.

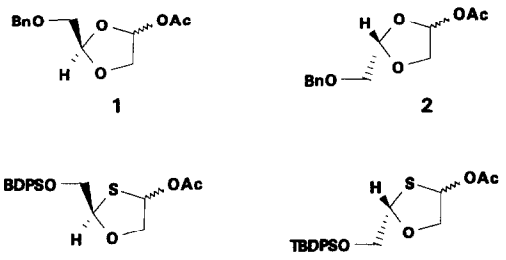
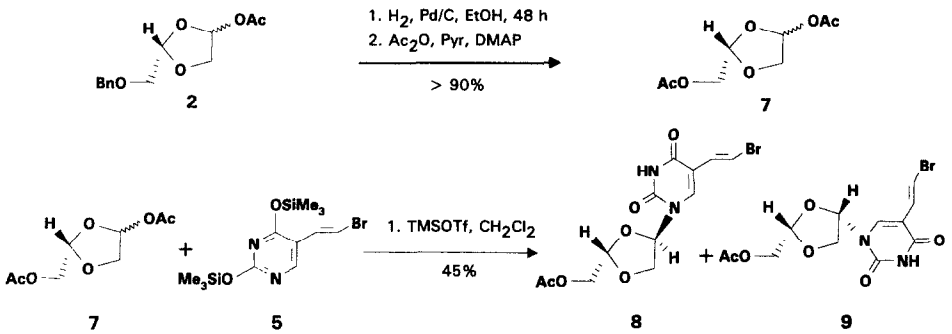
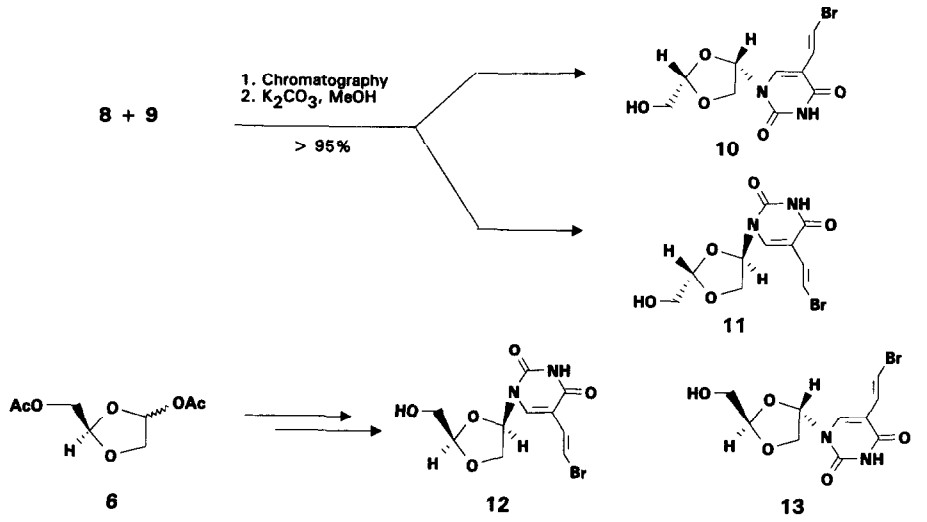


Figure 1. Structure of sugar precursors 1 - 4.

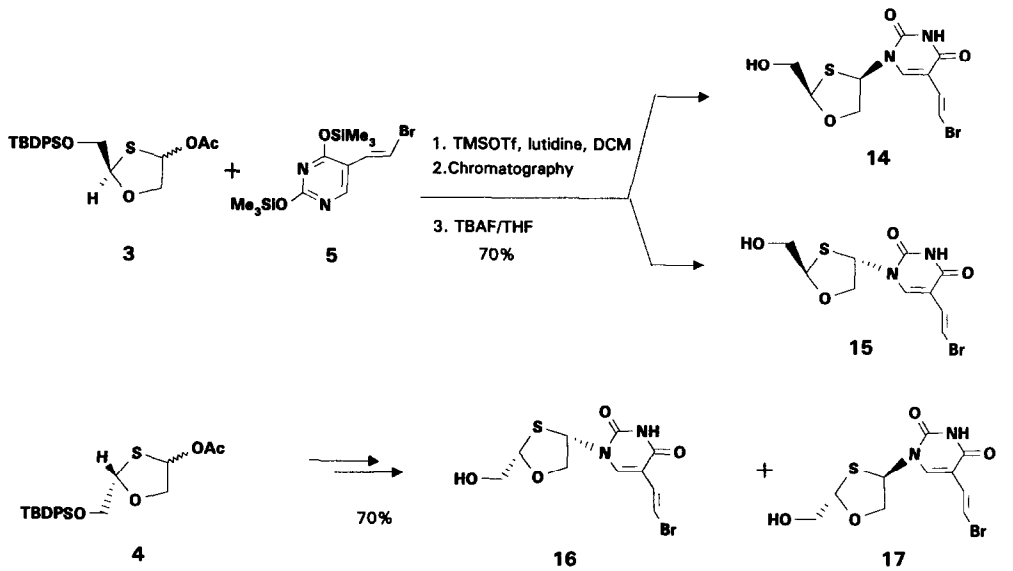
Dioxolanes **1** and **2** were prepared in six steps from readily available L-ascorbic acid.[5] Conversion of **1** to the diacetate **6** likewise followed standard procedures of transfer hydrogenolysis and acylation (cyclohexene, PdO then Ac_2O/pyr, 40% yield).[5] A substantial increase in the yield of **6** was obtained by debenzylation with hydrogen over Pd on charcoal and acylation. In this manner, **6** and **7** were prepared in 90% yield. Treatment of **7** with **5** led to the diastereomeric nucleosides **8** and **9** (1.2:1 ratio) in 50% yield. Following chromatographic separations, each of **8** and **9** were hydrolyzed to afford the 2S,4S and 2S,4R isomers **10** and **11**, respectively, in excellent yield for biological evaluation (Scheme 1).[19]





Scheme 1. Synthesis of dioxolanes 10 - 13

The isomeric purity of the final compounds was assessed by analytical chiral HPLC analyses on YMC PVA-SIL 5μ 120 Å column using EtOAc-hexanes (1:1) solvent mixtures. Recognizing the efficiency of this route, we prepared nucleosides **12** and **13** from **6** in an analogous manner and adapted this methodology to the preparation of the oxathiolane derivatives **14-17** (Scheme 2).



Scheme 2. Synthesis of oxathiolane nucleosides 14-17

The anti-herpetic activities and cytotoxicities of nucleosides **10-17** were determined in plaque reduction assays in vero cells and Flow 2002 (human fibroblast) cells infected with HSV-1 (KOS strain), HSV-2 (186 strain) and HCMV (WFI strain), respectively.[20] The results are shown in Table 1, together with those of acyclovir (ACV) and ganciclovir (DHPG) as references.

Table 1. Activities of Nucleosides 10-17 Against Herpes Viruses.

Compound	IC_{50} μg/ml HSV-1	IC_{50} μg/ml HSV-2	CC_{50} μg/ml, Vero Cells	IC_{50} μg/ml HCMV	CC_{50} μg/ml, Flow 2002 Cells
10	0.3	>30	100	5	30
11	3.4	>30	100	>30	30
12	>30	>30	100	>100	>100
13	>30	>30	100	25	100
14	>30	2.9	100	>100	100
15	>10	>10	30	80	100
16	>100	>100	>100	>100	100
17	>30	30	100	>100	>100
ACV	0.10	0.50	>10	---	---
DHPG	---	---	---	0.1	>100

Taking into account the differences of absolute and relative stereochemistry of the nucleosides and sulphur versus oxygen substitution in the sugar ring, the results indicate several intriguing trends. First, dioxolane **11** with α-L configuration displayed moderate inhibitory activity against HSV-1, while its sulphur analogue **17** was weakly inhibitory to HSV-2 replication. The α-D derivatives **13** and **15** were weakly active against HCMV replication. Second, oxathiolane **14** emerged as the most potent anti-HSV-2 analogue in this series being six-fold less potent than ACV. The mere replacement of sulphur by oxygen in **12** abolished the activity against HSV-2. Third, nucleoside **10** emerged as a potent agent against HSV-1 replication being 3-fold less potent than ACV, and was active against HCMV replication at concentrations below the cytotoxic dose. Interestingly, by contrast its sulphur analogue **16** was devoid of activity.

The mode of action of anti-herpetic BVU containing nucleosides has been extensively studied.[21] Specific phosphorylation by the virus-encoded thymidine kinases (TK) converts them to their 5'-monophosphates (MP) in infected cells. The HSV-1 TK, but not HSV-2 TK, is associated with MP kinase activity thus causing further conversion to the diphosphates and eventually to the 5'-triphosphates.[22] The lack of HSV-2 encoded TK activity explains the inactivity against HSV-2 observed in **10**, **11**, **12**, **13**, **15** and **16**. At the present time, we have no compelling explanation for the activity of **14** or even **17**, against HSV-2 and attribute that to the structural features as a consequence of heteroatom substitution. The potency of **10** and **11** represent, to the best of our knowledge, the first examples of β-L and α-L 2',3'-dideoxynucleoside analogues with activity against HSV-1 *in vitro* and broaden the substrate specificity of the HSV-1 TK, resulting from heteroatom substitution in the sugar ring.

Concerning the relationship between absolute configuration and anti-herpetic activity of BVU nucleosides, Balzarini *et al.* reported that in carbocyclic BVDU analogues, the activity resided primarily in the β-D "natural" enantiomer.[23] In this work, the anti-HSV-1 activity resided entirely in the L-isomers (β- and -α) of dioxolanes **10** and **11**, which represents an unexpected and unique case in the nucleoside field.

In summary, we have described the first examples of anti-herpetic activities in the heteroatom substituted series of 2',3'-dideoxynucleoside analogues, and have shown that the stereochemical requirement for activity is not readily predicted on the basis of literature precedence and that sulphur versus oxygen substitution causes substantial difference in antiviral profile. More importantly, we have demonstrated that heteroatom substitution in the ring[24] mimics the 3'-α-hydroxymethine (CHOH) moiety, native to nucleosides.[25]

Acknowledgement

The authors wish to thank Dr. R. Storer of Glaxo Research and Development Ltd. for useful discussions, the analytical sciences group at BioChem for HPLC separations and optical purity determination, as well as Dr. J. Gillard and colleagues for encouragement.

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24. (±)-2'-Deoxy-3'-thia-(E)-5(2-bromovinyl)uridine IC_{50} (HSV-1) 3.88 µg/ml. Interestingly, this nucleoside carries a 3'-thia moiety.
25. Satisfactory spectral and analytical data were obtained and will be reported elsewhere. Selected physical data: **10** (BCH-2639) mp 176-178°C; $[\alpha]^{25}_D$ - 4.6°(c 0.28, MeOH) **11** mp 77-79°C; $[\alpha]^{25}_D$ + 3.2° (c 0.23, MeOH) **12** mp 178-180°C; $[\alpha]^{25}_D$ + 4.8°(c 0.24, MeOH) **13** mp 76-78°C; $[\alpha]^{23}_D$ - 3.9°(c 0.86, MeOH) **14** (BCH-2647) mp 163-165°C; $[\alpha]^{25}_D$ - 41.6°(c 0.51, MeOH) **15** mp 134-136°C; $[\alpha]^{25}_D$ + 118.8°(c 0.50, MeOH) **16** mp 161-163°C; $[\alpha]^{25}_D$ + 40.0°(c 0.50, MeOH) **17** mp 134-136°C; $[\alpha]^{25}_D$ - 116.8°(c 0.50, MeOH).

(*Received in USA* 22 *August* 1994; *accepted* 6 *October* 1994)