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Antiviral Optically Pure Dioxolane Purine Nucleosides Analogues

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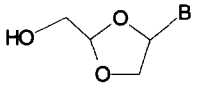
Abstract: Selective deamination of (±) cis 2,6-diaminopurine dioxolane nucleoside produces the (-) guanine analogue having the 2R,4R absolute stereochemistry. The (±) cis-adenine analogue generates the 2R,4R hypoxanthinyl derivative. Asymmetric synthesis of purine dioxolanes have been developed. The (-) adenine and (-) guanine compounds **6** and **7** emerged as potent inhibitors of the HIV-1 replication *in vitro*.

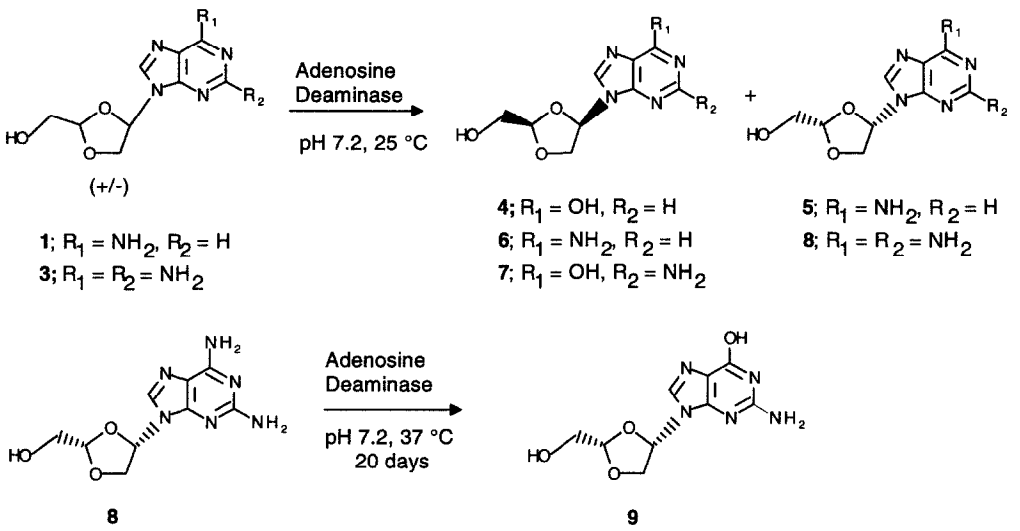
There has been considerable recent interest in the design and synthesis of nucleoside analogues as potential antiviral agents.[1] Several dideoxynucleosides emerged as potentially effective therapeutic agents for the inhibition of the replication of the human immunodeficiency virus (HIV), the causative agent of AIDS, however, toxicity and resistance problems posed by the compounds in the clinic have prompted the need to search for new agents with improved pharmacological profiles.[2]

Recently, Belleau et al. reported the synthesis and HIV inhibitory effects of dideoxynucleoside prototypes containing a 3'-thia and 3'-oxa heteroatoms in the carbohydrate moiety.[3] Chiral syntheses and anti-HIV activity of pyrimidine analogues have also appeared.[4,5] In this communication, we report the enzymatic resolution, chiral synthesis and biological evaluation of purine dioxolane analogues with the aim of identifying suitable antiviral agents.[6]



1; B = adenine
2; B = guanine
3; B = 2,6-diaminopurine

Enzymatic routes to the enantiomers of **1** and **2** were considered since the racemic analogues exhibited biological activities. Thus, the action of adenosine deaminase[7] (ADA) in 0.05M phosphate buffer (pH 7.2, 25°C) on **1** generated **4** which was readily separated from **5** by reverse phase HPLC techniques. Chiral HPLC analysis confirmed the complete deamination of **6** to afford **4** after 24 hours and that **5** proved to be resistant to further deamination by ADA for an extended period of time. Following the protocol of Vince and Brownell[8], compound **3** furnished **7** and **8** after 24 hours, which were readily separated by HPLC techniques.[9] Furthermore, compound **8** was converted to **9** upon prolonged exposure to ADA (37 °C, 500 hr) as depicted in Scheme 1.

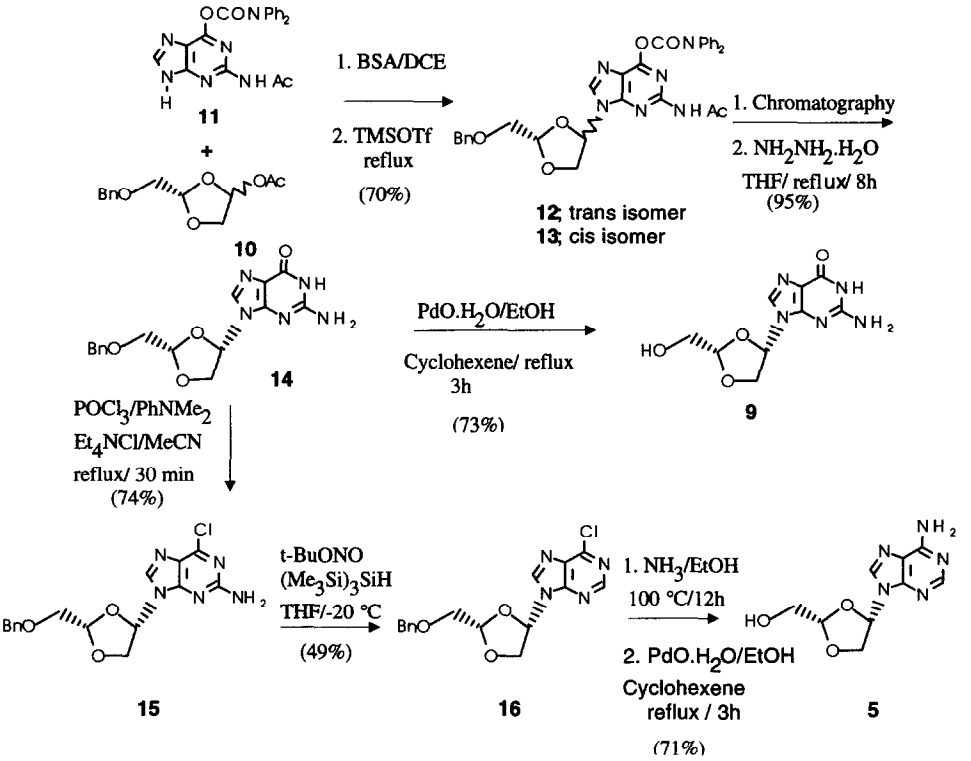


Scheme 1. Enzymatic Resolution of Racemic Purine Dioxolane Nucleosides

Although, ADA is known to tolerate variations in the carbohydrate framework of nucleosides[10], its ability to selectively recognize one enantiomer of **1** and **3** provides for the rapid preparation of analogues needed for biological testing.

The absolute stereochemistry of **7**, **8** and **9** was established by synthesis. Dioxolane **10**[5] was coupled with silylated diphenylcarbamoylpurine derivative **11** under reflux[11] with trimethylsilyltriflate to furnish a 1:1 mixture of the N-9 regioisomers **12** and **13**. After separation, Compound **13** was deprotected with hydrazine hydrate[12] to give the guanine derivative **14** in good yield. Removal of the benzyl group by transfer hydrogenolysis afforded pure **9** (94% ee, Chiral HPLC) which possesses the 2S,4S absolute configuration (Scheme 2). In a similar fashion, compound **7** was prepared from the 2R epimer of **10**. By comparing the analogues obtained by the chemical and enzymatic routes, it can be concluded that ADA selectively recognized the ß configuration of **3**.

The stereochemistry of **5** was established by guanine to adenine interconversion achieved in several steps. First, phosphorous oxychloride-tetraethylammonium chloride mediated chlorination[13] of **14** afforded **15** in good yield. Reductive deamination of **15** was achieved by reaction with t-butylisonitrite followed by the addition of tris(trimethylsilyl)silane[14] in THF to produce **16** which was sequentially aminated, deprotected and purified to give the adenine analogue **5** having the 2S,4S configuration (Scheme 2).



Scheme 2. Syntheis of (+)2S,4S Guanine and Adenine Dioxolane Analogues

The nucleoside analogues **5,6,7** and **9** were tested for inhibitory activity against HIV in whole cell assay (MT-4, RF strain of HIV-1 at concentration up to 425 μM). Compound **6** showed excellent activity (IC_{50} = 1.3 μM) and was weakly cytotoxic (CD_{50} = 425 μM), whereas **7** exhibited good activity (IC_{50} = 10.7 μM) and was not cytotoxic (CD_{50} > 425 μM). Analogues **5** and **9** were inactive and not cytotoxic.

Our biological data is not in total agreement with the recently reported activities of **6** (IC_{50} = 0.5 μM; CD_{50} >100 μM) and **7** (IC_{50} = 0.03 μM; CD_{50} >100 μM) determined in human peripheral blood mononuclear (PBM, LAV strain of HIV-1) cells[15] due to the difference in phosphorylation or transport in these two cell lines.

In summary, we have shown that dioxolane, adenine and guanine nucleosides are substrates for ADA which selectively deaminates the ß - analogues, and have achieved a stereoselective synthesis of these analogues. The anti-HIV activity residing in the ß -analogues **6** and **7** warrants further investigations of this novel class of compounds.

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9. **6** m.p. 247-248°C α_D - 29° (C 0.1, MeOH) ^{13}C NMR 75.4 MHz (DMSO) δ 61.2, 70.7, 79.4, 105.8, 118,8, 138.9, 149.6, 153.2, 156.2, for details of obtaining **6** by HPLC resolution see accompanied reference. **7** m.p. 270°C α_D -63.2° (C 0.22,H_2O)^{13}C NMR 75.4MHz (DMSO) δ 61.2, 70.6, 78.8, 105.7, 116.4, 135.0, 151.2, 154.2, 157.1. ^1H NMR data are in agreement with those reported in detail in reference 15. The optical purity was established by chiral HPLC analyses see DiMarco, M.P. Evans, C.A., Dixit, D.M., Brown, W.L., Siddiqui, M.A., Tse, H.L.A.,Jin, H., Nguyen-Ba, N. and Mansour, T.S., J. Chromatography (in press) .
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11. Zou, R. and Robins, M.J., *Can. J. Chem.* **1987**, 65, 1436. Interestingly, no N-7 regioisomers were detected by TLC and ^1H NMR of the crude reaction mixture after workup. **12** ^1H NMR ($CDCl_3$) δ 2.51 (s,3H), 3.64 (d,2H,J=3Hz), 4.48 (m,2H), 4.63 (s,2H),5.68 (t,1H,J=3Hz), 6.42 (dd,1H,J=3Hz and 5Hz), 7.25-7.44 (m,15H), 8.08 (s,1H), 8.20 (br s,1H,D_2O exchangeable). **13** ^1H NMR ($CDCl_3$) δ 2.53 (s,3H), 3.74 (d,2H,J=3Hz), 4.25 (dd,1H,J=5Hz and 10Hz), 4.45 (dd,1H,J=10Hz), 4.61 (s,2H), 5.23 (t,1H,J=3Hz), 6.45 (d,1H,J=4Hz), 7.23-7.45 (m,15H), 8.22 (br s,1H,D_2O exchangeable), 8.38 (s,1H).
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