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ANTI-HUMAN IMMUNODEFICIENCY AND ANTI-HEPATITIS B VIRUS ACTIVITIES OF β-L-2',3'-DIDEOXY PURINE NUCLEOSIDES

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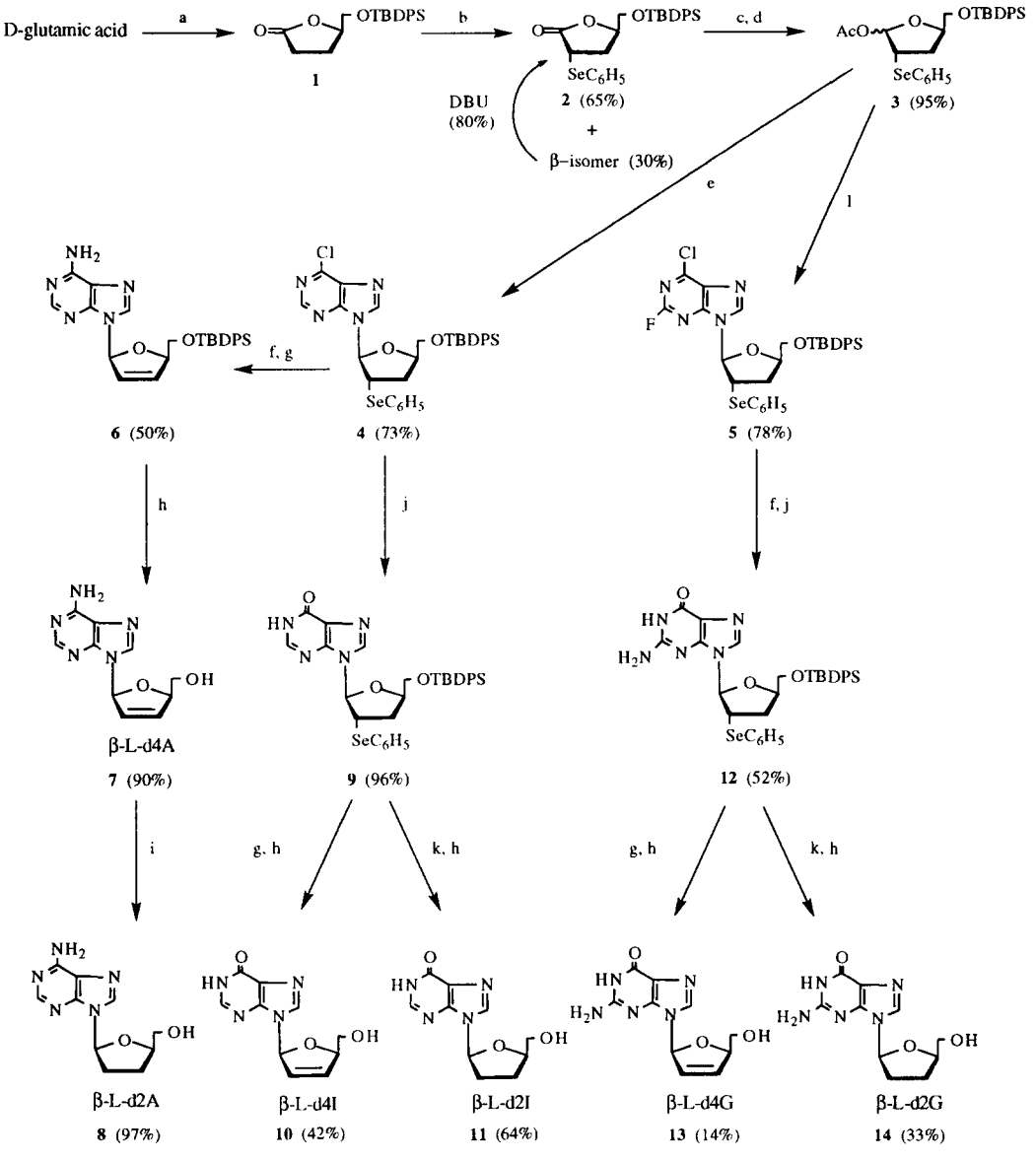
Abstract. β-L-2',3'-Dideoxyadenosine, β-L-2',3'-didehydro-2',3'-dideoxyadenosine and related compounds were synthesized in a stereoselective manner. These compounds were tested in vitro against HBV in 2.2.15 cell line and against HIV-1 in PBM and CEM cells. It was found that β-L-2',3'-didehydro-2',3'-dideoxyadenosine (**7**) exhibited significant anti-HIV (EC_{50} 0.38 μM in PBM cells) and anti-HBV activity (EC_{50} 1.2 μM).

Pandemic morbidity and mortality due to the human immunodeficiency virus (HIV) and hepatitis B virus (HBV) have been responsible for the intensive efforts in discovering more effective and less toxic antiviral agents against these viruses. HIV and HBV each encode their own reverse transcriptase,[1-3] which are requisite enzymes in their replicative cycles. Currently, five nucleoside reverse transcriptase inhibitors (AZT, ddC, ddI, d4T and 3TC) and three protease inhibitors (saquinavir, ritonavir and indinavir) are available for the treatment of AIDS, although other viral targets have been investigated.[4] In recent years, drug resistance[5,6] and toxicity of the existing regimens have prompted the development of additional anti-HIV agents to circumvent these drawbacks. Additionally, despite over 300 million HBV chronic carriers, no effective and safe chemotherapeutic agents are available today for the treatment of these patients.

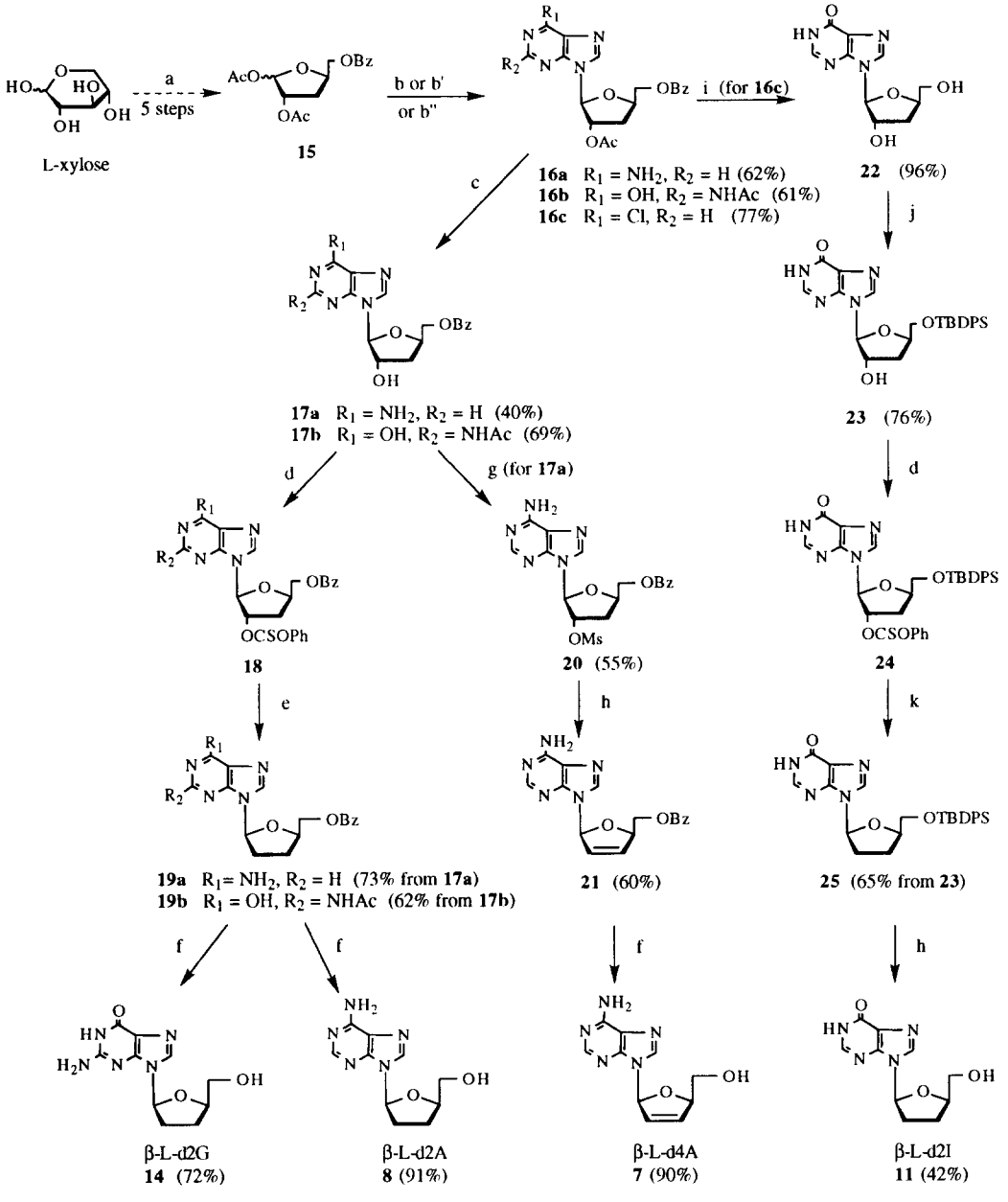
Previously, β-D-2',3'-dideoxynucleosides (ddN) and β-D-2',3'-didehydro-2',3'-dideoxynucleosides (d4N) have been shown to possess significant anti-HIV activity.[7] Of the purine nucleosides, β-D-2',3'-dideoxyadenosine (D-ddA) and β-D-2',3'-didehydro-2',3'-dideoxyadenosine (D-d4A) exhibited significant anti-HIV activity. However, D-ddA is susceptible to the action of adenosine deaminase[8] (ADA) and purine nucleoside phosphorylase[9] (PNP), leading to inactive metabolites.

Scheme 1. Synthesis of various β-L-d4N and β-L-d2N nucleosides from D-glutamic acid.



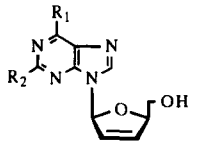
(a) (i) $NaNO_2$, HCl, rt; (ii) BH_3, THF, rt; (iii) TBDPSCl, Imidazole, CH_2Cl_2, rt; (b) (i) LiHMDS, -78 °C, THF; (ii) TMSCl, rt; (iii) PhSeBr, -78 °C, THF; (c) DIBAL-H, -78°C, toluene; (d) Ac_2O, DMAP, CH_2Cl_2, 0 °C; (e) TMS-6-chloropurine, TMSOTf, $ClCH_2CH_2Cl$, -22 °C; (f) NH_3/MeOH, 80 °C; (g) H_2O_2, pyridine, CH_2Cl_2, 0 °C; (h) 1M TBAF/THF, rt; (i) 15 psi H_2, 10% Pd/C, rt; (j) $HSCH_2CH_2OH$, NaOMe, MeOH, reflux; (k) Bu_3SnH, Et_3B, benzene, rt; (l) TMS-6-chloro-2-fluoropurine, TMSOTf, $ClCH_2CH_2Cl$, 0 °C.

Scheme 2. Synthesis of various β-L-d4N and β-L-d2N from L-xylose.

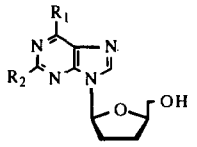


(a) Ref 25; (b) 2 eq $SnCl_4$, adenine, CH_3CN, rt; (b') TMS-N^2-acetylguanine, TMSOTf, CH_3CN, reflux; (b") TMS-6-chloropurine, TMSOTf, $ClCH_2CH_2Cl$, rt; (c) NaOMe, THF, rt; (d) PhOCSCl, DMAP, CH_2Cl_2, rt; (e) $(Me_3Si)_3SiH$, AIBN, dioxane, rt; (f) NH_3/MeOH, rt; (g) 3 eq MsCl, pyridine, rt; (h) 1M TBAF/THF, rt; (i) $HSCH_2CH_2OH$, NaOMe, MeOH, reflux; (j) TBDPSCl, pyridine, rt; (k) $(Me_3Si)_3SiH$, AIBN, dioxane, reflux.

Table 1. Anti-HIV and anti-HBV activities of various β-L-d4N and β-L-d2N's.



7 $R_1 = NH_2$, $R_2 = H$
10 $R_1 = OH$, $R_2 = H$
13 $R_1 = OH$, $R_2 = NH_2$



8 $R_1 = NH_2$, $R_2 = H$
11 $R_1 = OH$, $R_2 = H$
14 $R_1 = OH$, $R_2 = NH_2$

	Anti-HIV-1 Activities			Anti-HBV Activities		
Compound	**Cell Line**	**EC_{50} (μM)**	**IC_{50} (μM)**	**Cell Line**	**EC_{50} (μM)**	**IC_{50} (μM)**
7 β-L-d4A	PBM CEM	0.38 0.54	59 37	2.2.15	1.2	70
8 β-L-d2A	PBM CEM	8.2 -	> 100 -	2.2.15	6.0	> 200
10 β-L-d4I	PBM CEM	5.5 -	60 -	2.2.15	> 10	50
11 β-L-d2I	PBM CEM	> 100 -	> 100 -	2.2.15	> 10	> 200
13 β-L-d4G	PBM CEM	14.1 18.1	> 100 > 10	2.2.15	> 10	50
14 β-L-d2G	PBM CEM	> 100 > 100	> 100 > 100	2.2.15	> 10	200

EC_{50} = concentration required to inhibit 50% of virus; IC_{50} = concentration required to inhibit 50% of host cells; - = not determined.

Recently, L-nucleosides have proven to be of great importance as anti-HIV[10-15] and anti-HBV[15-19] agents with a number of these compounds showing promising activity when compared to the corresponding D-nucleosides. It has been reported that the β-L-compounds (–)-*cis*-5-fluoro-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine (FTC) and (–)-*cis*-1-[2-(hydroxymethyl)-1,3-dioxolan-4-yl]cytosine (OddC) were resistant against cytidine deaminase[18,20-22] unlike their D-counterparts, which may be related to their more potent anti-HIV and anti-HBV activity. Consequently, it was of interest to synthesize the corresponding β-L-enantiomers of D-ddA and D-d4A as potential anti-HIV and anti-HBV agents in anticipation that these compounds may be resistant to the degradative enzymes ADA and PNP. As a part of our drug discovery efforts for antiviral agents, herein we report

preliminary anti-HIV and anti-HBV activity of several β-L-purine nucleosides.

Synthesis. The synthesis of new purine β-L-d2N and β-L-d4N analogs is described utilizing two approaches (Schemes 1 and 2). Lin et al have also recently reported the synthesis of some purine L-d2N using different methodology.[23] Scheme 1 describes the syntheses of both β-L-d4N and β-L-d2N from the stereoselectively prepared 3-α-phenyl selenium intermediate **2**.[24] Reduction and acetylation of **2**, followed by coupling with various silylated bases provided intermediates **4** or **5** that were converted to either the unsaturated L-d4N (via an oxidative elimination process) or the saturated L-d2N nucleosides (via a radical reductive process). For example, treatment of **9** with H_2O_2 in the presence of a catalytic amount of pyridine, followed by deprotection provided the unsaturated inosine derivative **10**. Compound **9** was treated with Bu_3SnH and Et_3B, followed by deprotection to provide the saturated inosine derivative **11**. Scheme 2 affords both L-d4N and L-d2N analogs from L-xylose, via the common intermediate **15**.[25] Coupling of **15** with various silylated bases, followed by selective deprotection of the 2'-hydroxyl functionality provided intermediates **17a**, **17b** or **22** that were converted to either the unsaturated L-d4N (via an elimination of the mesylate) or saturated L-d2N nucleosides (via a radical reductive process). For example, treatment of **17a** with phenyl chlorothionoformate and dimethylaminopyridine allowed for a subsequent radical deoxygenation of **18** with tris(trimethylsilyl)silane to afford **19a**, which upon deprotection provided the desired saturated adenosine analog **8**. Unsaturated nucleosides were also obtained by the treatment of **17a** with mesyl chloride and pyridine to obtain **20**, which in the presence of 1 M tetrabutylammonium fluoride in THF, yielded the unsaturated intermediate **21**, and subsequent deprotection afforded the desired unsaturated adenosine analog **7**.[26]

Antiviral activity. Both β-L-d4N (**7**, **10** and **13**) and β-L-d2N (**8**, **11** and **14**) were evaluated against HIV and HBV. The antiviral testing against HIV were performed in human peripheral blood mononuclear (PBM) cells and a CEM cell line. The β-L-d4N were generally more active against HIV, while more toxic than their β-L-d2N counterparts. Within the class of β-L-d4N, the adenine analog **7** was more potent than either the hypoxanthine **10** or the guanine analog **13**. The adenine analog **7** exhibited a similar toxicity profile when compared to the hypoxanthine analog **10**. The class of β-L-d2N displayed a similar activity profile with the adenine analog **8** being more active than either the hypoxanthine **11** or the guanine **14** analogs, while toxicity data were similar for all three compounds against HIV. Against HBV, differences between β-L-d4N and β-L-d2N activities and toxicities were marginal as were differences between activities and toxicities due to different nucleoside bases. The adenine analogs **7** and **8** were generally more potent than either the hypoxanthine **10** and **11** or guanine analogs **13** and **14**.

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26. Data for **7**: mp 187-188 °C; UV (MeOH) λ_{max} (pH 7) 259.5 (ε 13363), (pH 2) 258 (ε 12975), (pH 11) 259.9 (ε 12981); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.59 (t, J = 4.6 Hz, 2H), 4.90 (m, 1H), 5.07 (t, J = 5.5 Hz, 1H), 6.15 (ddd, J = 1.5, 1.8, 5.9 Hz, 1H), 6.48 (ddd, J = 1.5, 1.8, 5.9 Hz, 1H), 6.95 (m, 1H), 7.30 (br s, 2H), 8.16 (s, 1H), 8.23 (s, 1H); $[\alpha]^{25}_D$ = 24.5 (*c* 0.5, DMSO); MS *m/e* $(M+H)^+$ 234; Anal. Calcd for $C_{10}H_{11}N_5O_2$: C, 51.49; H, 4.75; N, 30.03. Found: C, 51.54; H, 4.77; N, 30.00. Compound **10**: mp >310 °C; UV (MeOH) λ_{max} (pH 7) 249 (ε 9440), (pH 2) 249 (ε 9530), (pH 11) 259 (ε 10210); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.58 (m, 2H), 4.93 (m, 2H), 6.16 (br d, J = 6.0 Hz, 1H), 6.51 (br d, J = 6.0 Hz, 1H), 6.92 (m, 1H), 8.09 (s, 1H), 8.13 (s, 1H); $[\alpha]^{27}_D$ = 35.0 (*c* 0.05, H_2O); MS *m/e* $(M+H)^+$ 235. Anal. Calcd for $C_{10}H_{10}N_4O_3$: C, 51.28; H, 4.30; N, 23.92. Found: C, 51.41; H, 4.33; N, 23.65.

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