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ENANTIOMERICALLY PURE SYNTHESIS AND ANTIVIRAL EVALUATION OF [(2'*S*, 3'*S*)-BIS(HYDROXYMETHYL)AZETIDIN-1-YL] PURINE NUCLEOSIDES: ANALOGS OF OXETANOCIN-A

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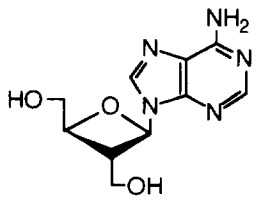
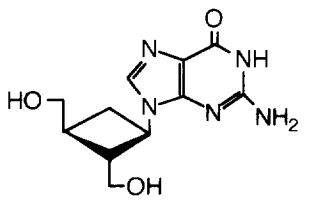
Abstract: The enantiomerically pure synthesis of [(2'*S*, 3'*S*)-bis(hydroxymethyl)azetidin-1-yl] adenine **9** and -guanine **13** was achieved *via* construction of the base on the 1-amino-azetidine **4** and their anti-HSV-1 and -2, and anti-HIV-1 activities were evaluated.

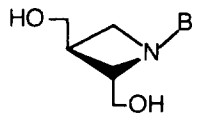
Various compounds have been reported as potent and selective inhibitors of human immunodeficiency virus (HIV) targeted at the virus-encoded reverse transcriptase. At present, besides AZT (3'-azido-3'-deoxythymidine), ddI (2',3'-dideoxyinosine) and ddC (2',3'-dideoxycytidine) being the only approved drugs for the clinical treatment of AIDS, some very recently prepared nucleosides such as d4T (2',3'-didehydro-3'-deoxythymidine) and (-)-3TC (β-L-(-)-2'-deoxy-3'-thiacytidine, Lamivudine™) show very promising anti-HIV activity and selectivity. However, it is critical to search for new and less toxic anti-HIV agents which are not cross-resistant with the existing drugs. As part of our continuing studies on the preparation and antiviral evaluation of analogs of Oxetanocin-A **1** and COXT-G **2**, we recently reported the first synthesis of [(2'*S*, 3'*S*)-bis(hydroxymethyl)azetidin-1-yl] pyrimidine nucleosides **3**.[1] In this report we describe the synthesis and antiviral activities of [(2'*S*, 3'*S*)-bis(hydroxymethyl)azetidin-1-yl] adenine **9** and -guanine **13**.



1 Oxetanocin-A

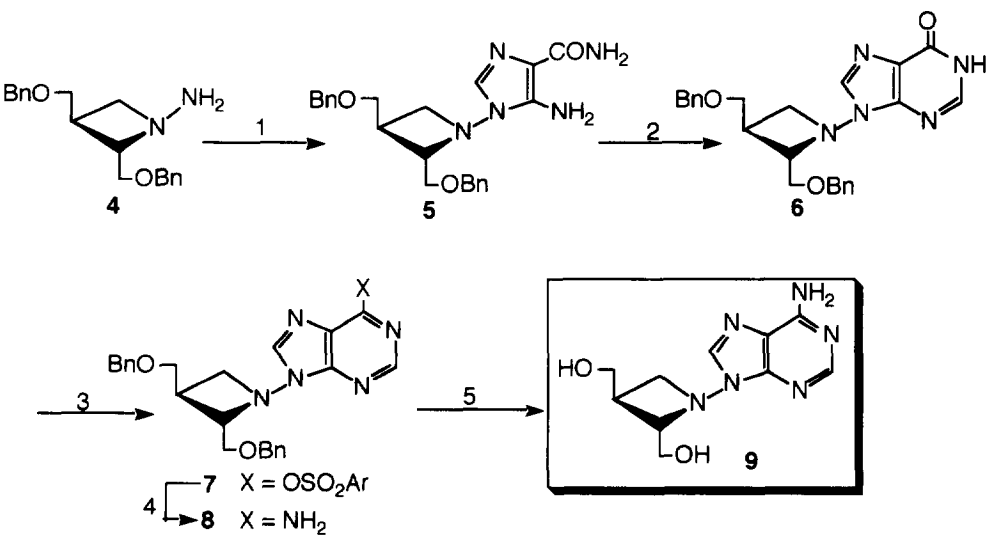


2 COXT-G



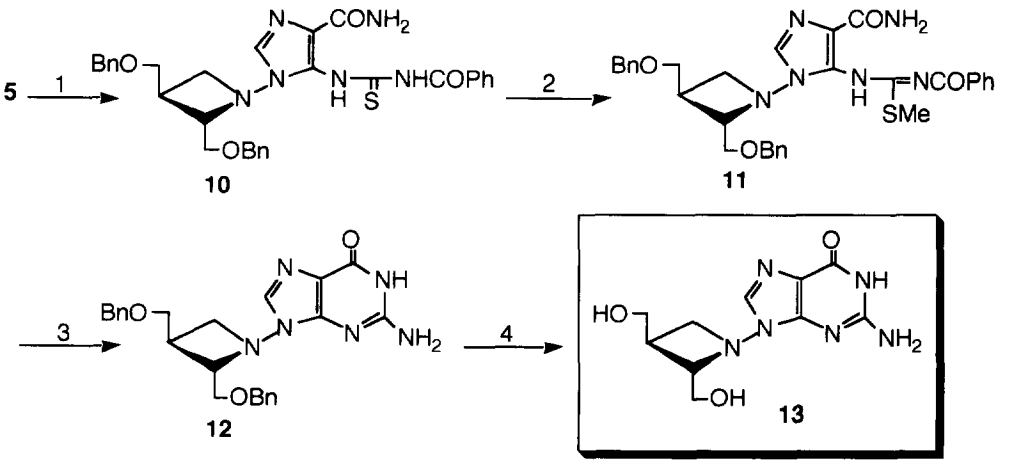
3 B = nucleic acid base

The key intermediate (2*S*, 3*S*)-*N*[1]-amino-2,3-bis(benzyloxymethyl)azetidine **4**[1] was progressed to purine derivatives *via* an imidazole intermediate.[2] Treatment of compound **4** with ethyl *N*-(carbamoylcyanomethyl)formimidate[3] gave the imidazole **5** in 42 % yield, which was converted into the hypoxanthine **6**[8] in 60 % yield using triethyl orthoformate[4]. Compound **6** was successfully transformed into the adenine **8** *via* ammonolysis of the 2,4,6,-triisopropylbenzenesulfonate **7**[5] in 35 % yield (2 steps). Finally, deprotection of **8** by transfer hydrogenolysis with 20 % $Pd(OH)_2$ on carbon and cyclohexene afforded the target compound **9**[9] in 74 % yield (Scheme 1).[12]



Scheme 1 *Reagents and Conditions*: 1) EtO-CH=N-CH(CN)$CONH_2$, EtOH, reflux, 30 min; 2) $HC(OEt)_3$, DMF,120 °C, 20 min; 3) 2,4,6-triisopropylbenzenesulphonyl chloride, Et_3N, DMAP, CH_2Cl_2, rt, 2 h; 4) NH_3, EtOH, sealed tube, 80 °C, 6 h; 5) 20 % $Pd(OH)_2$/C, cyclohexene, EtOH, reflux, 5h.

To obtain the guanine derivative, the imidazole **5** was reacted with benzoyl isothiocyanate[6] to give the thiourea **10** in 74 % yield, which with methyl iodide in dilute sodium hydroxide yielded the methylthio derivative **11** in 95 % yield. Treatment of compound **11** with 2N-NaOH under reflux for 3h followed by neutralization of the reaction mixture with 2N-HCl provided the guanine **12**[10] in 68 % yield. Deprotection of **12** by transfer hydrogenolysis with 20 % $Pd(OH)_2$ on carbon and cyclohexene afforded the target compound **13**[11] in 61 % yield (Scheme 2).



Scheme 2 *Reagents and Conditions*: 1) PhCONCS, CH_3CN, reflux, 2 h; 2) 0.1 N-NaOH, MeI, rt, 2 h; 3) 2N-NaOH, MeOH, reflux, 3 h; 4) 20 % $Pd(OH)_2$/C, cyclohexene, EtOH, reflux, 5 h.

Antiviral Activity: The newly synthesized compounds **9** and **13** were evaluated *in vitro* against HSV-1 and HSV-2 in Vero cells by a plaque reduction assay and HIV-1 in MT-4 cells by an indirect immunofluorescence assay, respectively (Table 1). *Compounds **9** and **13** were found to demonstrate potent anti-HIV-1 activity at approximately the same level as ddI with EC_{50} values of 0.37, 0.46, and 0.46 μg/ml, respectively.* It is also noticeable that **13** was found to be 6-fold more active against HSV-1 and -2 than **9**, but was 16-fold less potent than acyclovir. Both **9** and **13** were devoid of cytotoxicity against Vero cells at concentrations up to 100 μg/ml.

Table 1 Antiviral activities of **9** and **13** against HSV-1, HSV-2, and HIV-1

	EC_{50} μg/ml[a]		
Compound	HSV-1	HSV-2	HIV-1
9	33.2	34.9	0.37
13	5.36	6.32	0.46
ddI	ND[b]	ND	0.46
AZT	ND	ND	0.0032
acyclovir	0.32	0.39	ND

a : Concentration required to inhibit HSV-1-, HSV-2-, or HIV-1-induced cytopathic effect by 50 %.

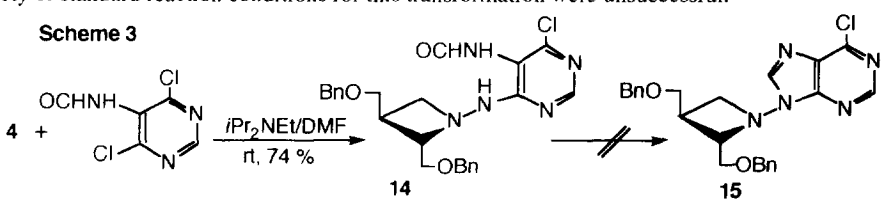
b : Not determined.

In conclusion, in a series of novel azetidin-*N*-nucleoside analogs, we have identified [(2'*S*, 3'*S*)-bis(hydroxymethyl)azetidin-1-yl] adenine **9** and -guanine **13** as good inhibitors of HIV-1 replication. This encouraging antiviral activity prompts us to study comprehensive structure-activity relationships for this class of compounds, which are in progress in our laboratories.

References and Notes:

1. Hosono, F.; Nishiyama, S.; Yamamura, S.; Izawa, T. and Kato, K. *Bioorg. & Medicinal Chem. Lett.*, **1994**, *4*, 2083-2086. Hosono, F.; Nishiyama, S.; Yamamura, S.; Izawa, T. and Kato, K. *Tetrahedron*, **1994**, *50*, 13335-13346 and see the references cited therein.
2. Initial attempts to synthesize the 6-chloropurine **15** from the azetidino-pyrimidine **14**, obtained from compound **4** and 4,6-dichloro-5-formamidopyrimidine, by closure of the imidazole ring under a variety of standard reaction conditions for this transformation were unsuccessful.[7]

Scheme 3



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7. Harnden, M. R.; Wyatt, P. G.; Boyd, M. R. and Sutton, D. *J. Med. Chem.*, **1990**, *33*, 187-196.
8. Selected spectroscopic data for **6**: colorless foam, ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.71 (1H, m), 3.60 (1H, dd, J=5.4 and 10.7 Hz), 3.63 (1H, dd, J=5.4 and 10.7 Hz), 3.80 (2H, d, J=6.4 Hz), 4.00 (1H, t, J=7.4 Hz), 4.47 (1H, d, J=12.7 Hz), 4.48 (1H, t, J=7.4 Hz), 4.58 (1H, d, J=12.7 Hz), 4.59 (2H, s), 4.82 (1H, m), 7.19~7.36 (10H, complex), 7.89 (1H, s), 8.13 (1H, s), and 13.04 (1H, broad, D_2O exchangeable); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ 31.9, 58.3, 70.8, 71.9, 72.2, 73.2, 73.3 , 117.3, 127.5 (x2), 127.6, 127.7 (x2), 127.8, 128.3 (x2), 128.4 (x2), 137.9, 138.1, 140.9, 144.3, 148.5, and 159.3; HRMS m/z 431.2034 calcd for $C_{24}H_{25}N_5O_3$, found 431.2013.
9. Selected spectroscopic data for **9**: colorless foam, $[\alpha]^{23}_D$ -25° (*c* 0.85, CH_3OH); λ_{max} (CH_3OH) 260 nm (ε 12,700); ^{1}H NMR (400 MHz, CD_3OD) δ 2.65 (1H, m), 3.59 (1H, dd, J=5.0 and 12.3 Hz), 3.65 (1H, dd, J=3.5 and 12.3 Hz), 3.88 (2H, d, J=5.8 Hz), 3.93 (1H, t, J=7.3 Hz), 4.39 (1H, t, J=7.3 Hz), 4.62 (1H, m), 8.20 (1H, s), and 8.24 (1H, s); ^{13}C NMR (100.5 MHz, CD_3OD) δ 34.1, 58.7, 63.5, 64.0, 75.2, 118.9, 142.2, 149.9, 153.4, and 157.5; HRMS m/z 250.1142 calcd for $C_{10}H_{14}N_6O_2$, found 250.1176.
10. Selected spectroscopic data for **12**: colorless foam, ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.68 (1H, m), 3.51 (1H, dd, J=4.4 and 12.0 Hz), 3.60 (1H, broad, D_2O exchangeable), 3.61 (1H, dd, J=5.1 and 12.0 Hz), 3.73 (2H, d, J=5.9 Hz), 3.92 (1H, t, J=7.4 Hz), 4.36 (1H, t, J=7.4 Hz), 4.47 (1H, d, J=12.3 Hz), 4.51 (1H, d, J=12.3 Hz), 4.53 (2H, s), 4.61 (1H, broad, D_2O exchangeable), 4.72 (1H, m), 6.43 (1H, broad, D_2O exchangeable), 7.18~7.34 (10H, complex), and 7.70 (1H, s); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ 31.8, 57.9, 70.6, 71.6, 71.7, 73.1, 73.3, 116.0, 127.4 (x2), 127.5, 127.6 (x2), 127.7, 128.3 (x2), 128.5 (x2), 137.5, 138.0, 138.2, 151.2, 153.3, and 159.3; HRMS m/z 446.2143 calcd for $C_{24}H_{26}N_6O_3$, found 446.2104.
11. Selected spectroscopic data for **13**: colorless foam, $[\alpha]^{25}_D$ -10° (*c* 0.38, H_2O); λ_{max} (H_2O) 255 nm (ε 13,200); ^{1}H NMR (400 MHz, D_2O) δ2.68 (1H, m), 3.70 (1H, dd, J=7.0 and 12.3 Hz), 3.74 (1H, dd, J=4.3 and 12.3 Hz), 3.90 (2H, d, J=5.9 Hz), 3.98 (1H, t, J=7.9 Hz), 4.11 (1H, t, J=7.9 Hz), 4.48 (m), and 8.18 (1H, s); ^{13}C NMR (100.5 MHz, D_2O) δ 33.7, 59.8, 63.3, 63.7, 75.3, 115.9, 139.2, 152.6, 155.2, and 160.6; HRMS m/z 251.1018 calcd for $C_{10}H_{13}N_5O_3$ (M^++H-NH_2), found 251.1042.
12. Detailed discussion of the theoretical and spectroscopic analysis of the conformation (*syn*/*anti*) around the pseudo-glycosidic bond of compound **9** will be disclosed in due course.

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