Syntheses of Oxanosine and Carbocyclic Oxanosine Derivatives as Anti-HIV Agents

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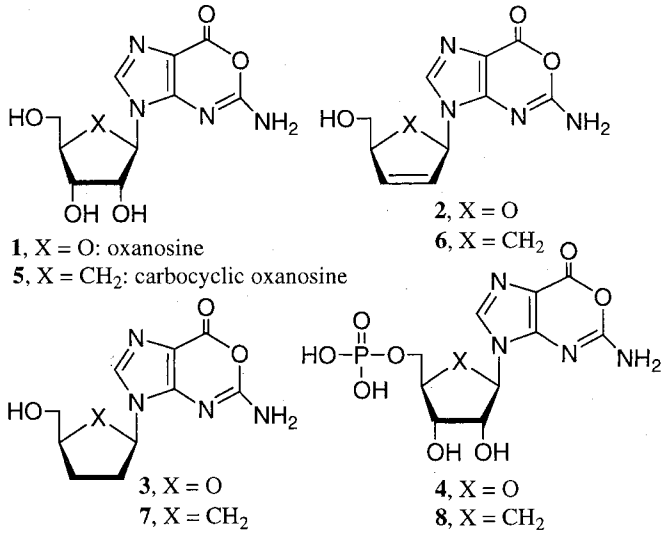
Since the discovery of AZT in 1985,[1] many kinds of sugar-modified nucleoside analogs have been synthesized for potential use as anti-HIV agents. At present, AZT, ddI, ddC, d4T, and (−)-3TC are approved for the clinical treatment of AIDS and AIDS-related complex, and several other sugar-modified nucleosides, *e.g.*, carbovir (carbocyclic 2′,3′-didehydro-2′,3′-dideoxyguanosine) are undergoing clinical or preclinical development.[2] However, it is still necessary to search for new anti-HIV agents that are more potent and less toxic. Moreover, drug-resistant HIVs continue to emerge rapidly.

Oxanosine **1**, a novel guanosine analog antibiotic isolated from the culture broth of *Streptomyces capreolus* MG265-CF3 has been reported to show antibacterial activity and to inhibit growth of HeLa cells in culture.[3] Furthermore, **1** has been proved to alter tumor cell morphology into normal morphology in temperature-sensitive K-ras transformed rat kidney (K-ras^{ts}-NRK) cells by inhibiting inosine monophosphate (IMP) dehydrogenase.[4] On the other hand, no anti-viral effect of oxanosine has been reported. In order to find new anti-HIV agents, we have synthesized a series of derivatives of oxanosine **1** and carbocyclic oxanosine **5**. In this report we describe the syntheses and structure-activity relationships of oxanosine and carbocyclic oxanosine derivatives as anti-HIV agents.

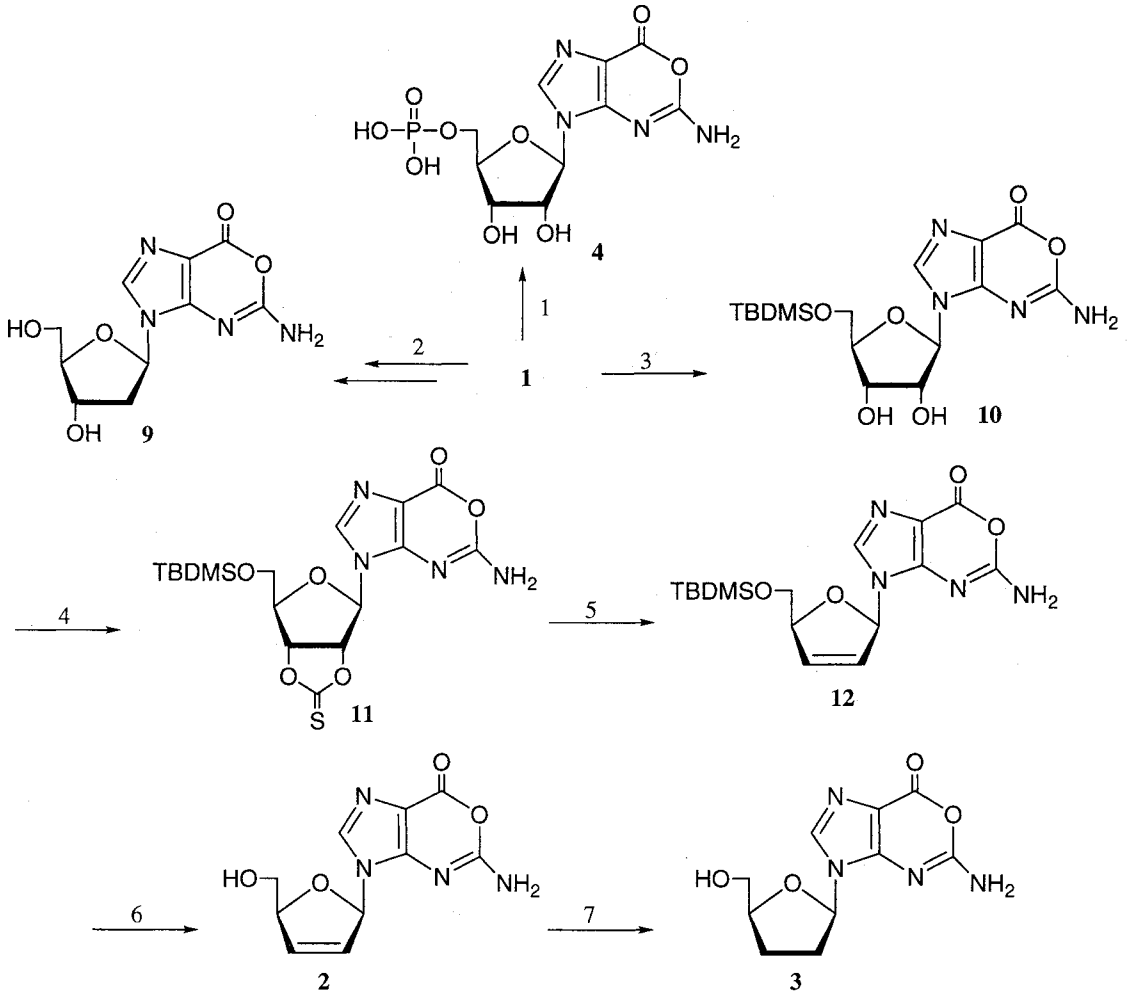
Individual steps leading to the target oxanosine derivatives are summarized in Scheme 1. After protection of the 5′-hydroxyl group of **1** with *t*-butyldimethylsilyl chloride, treatment of **10** with thiocarbonyldiimidazole in dichloroethane afforded thiocarbonate **11**, which was then refluxed with trimethyl phosphite to provide olefin **12**.[5] Deprotection of **12** with nBu$_4$NF gave 2′,3′-didehydro-2′,3′-dideoxyoxanosine **2** in 84% overall yield. Compound **2** was hydrogenated over 10% Pd/C in MeOH to give 2′,3′-dideoxyoxanosine **3**. Oxanosine 5′-monophosphate **4** could be easily obtained from **1** by treatment with phosphoryl chloride in trimethyl phosphate.[6] 2′-Deoxyoxanosine **9** was prepared from **1** in 4 steps according to known procedures.[7]

The synthetic route used to prepare carbocyclic oxanosine and its analogs is illustrated in Scheme 2. (−)-2-Azabicyclo[2,2,1]hept-5-en-3-one **13** was first converted to the Boc compound **14** according to the protocol of HUTCHINSON *et al.*[8] When compound **14** was treated with chloromethyl methyl ether in the presence of *N*,*N*-diisopropylethylamine, methoxymethyl compound **15** was obtained in 88% yield. Hydrolysis of **15** with H_2O[8] followed by treatment with ethyl *N*-(ethoxycarbonylcyanomethyl) formimidate[9] furnished the imidazole **16** in 62% yield in 2 steps. Then, compound **16** was reacted with ethoxycarbonyl isothiocyanate to give the thiourea **17** in 93% yield, which with methyl iodide in 0.1 N sodium hydroxide yielded the methylthio derivative **18** in 99% overall yield. Cyclization of compound **18** with 5 N-methanolic KOH under reflux for 30 minutes followed by neutralization of the reaction mixture with 4 N-HCl afforded

Fig. 1. Structure of oxanosine and carbocyclic oxanosine derivatives.



Scheme 1.



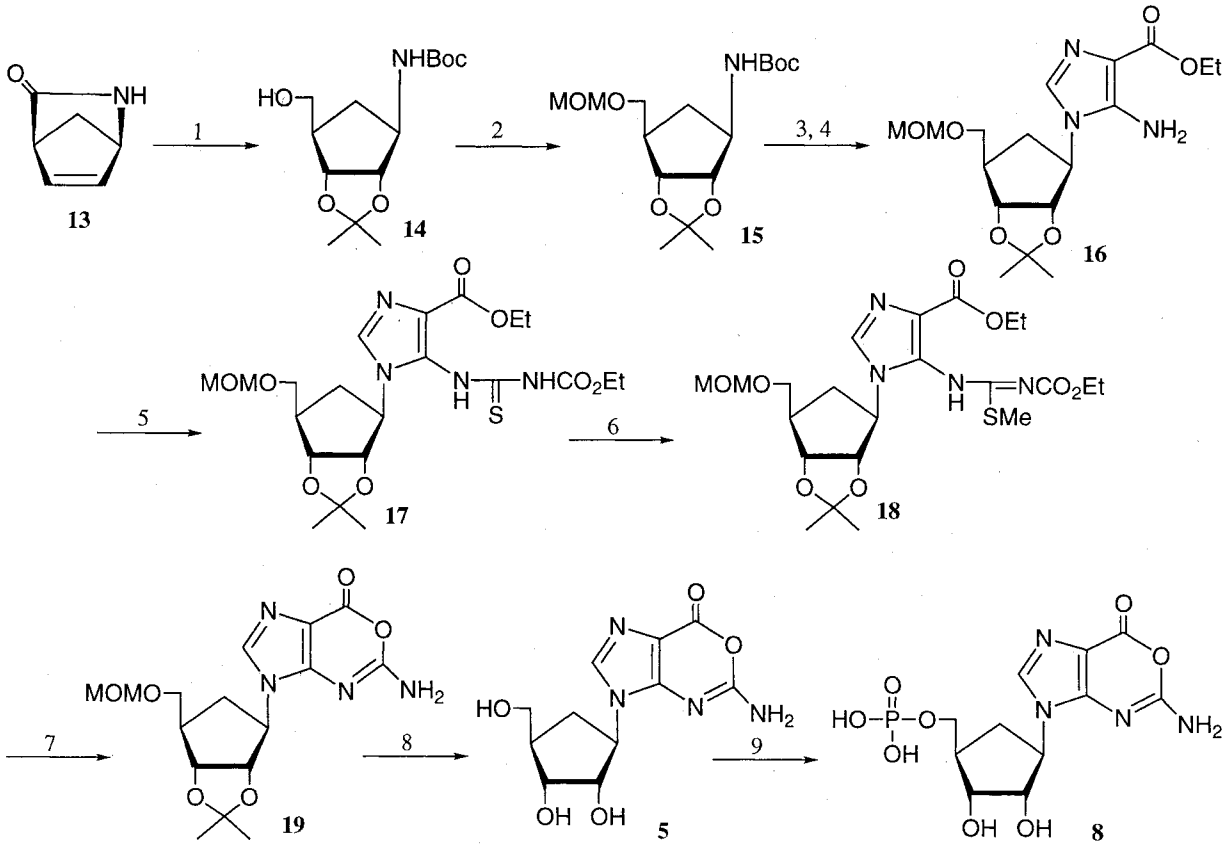
Reagents and conditions: 1) $PO(OMe)_3$, $POCl_3$, 0°C, 2 hours, 93% yield; 2) 4 steps see ref. 7; 3) TBDMSCl, pyridine, rt, 2 hours, 90%; 4) TCDI, $ClCH_2CH_2Cl$, rt, 4 hours, 67%; 5) $P(OMe)_3$, 120°C, 1.5 hours, 76%; 6) nBu$_4$NF, THF, rt, 1 hour, 84%; 7) H_2, 10% Pd/C, MeOH, rt, 3 hours, 81%.

imidazo-oxazinone **19** in 45% yield. Carbocyclic oxanosine **5** could be prepared from compound **19** by treatment with aqueous trifluoroacetic acid in 99% yield. Carbocyclic oxanosine 5′-monophosphate **8** was prepared from **5** by the same method described for **4** in 44% yield.

By employing similar reactions to those described for compound **5** from **13**, we obtained carbovir-type oxanosine analog **6** from compound **20** in 5 steps. Under neutral hydrogenation conditions with 10% Pd/C in EtOH, compound **6** afforded dideoxy carbocyclic oxanosine **7** in 92% overall yield.

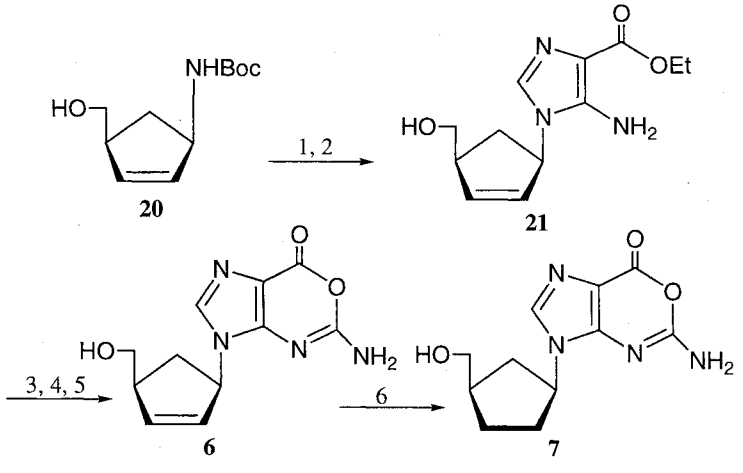
The anti-HIV-1 activity and cytotoxicity of the newly synthesized oxanosine and carbocyclic oxanosine derivatives were evaluated *in vitro* on human T cell leukemia CEM, H9, and U937 cell lines as shown in Table 1. The former two were used for an acute infection assay to evaluate the effect on HIV replication, especially the early steps in the infection process, and the latter, for a chronic infection assay to evaluate the decrease in HIV production from cells whose infection had already been established. In an acute assay CEM cells were more sensitive than H9 cells to AZT and oxanosine derivatives. Oxanosine itself showed anti-HIV activity in acute and chronic assays. Compounds **4** and **9** were found to demonstrate stronger acute anti-HIV activity than oxanosine in CEM cells with EC_{50} values of 4.1 and 4.8 μg/ml, respectively, without cytotoxicity up to

Scheme 2.



Reagents and conditions: 1) 4 steps see ref. 8; 2) $ClCH_2OCH_3$, *N,N*-diisopropylethylamine, CH_2Cl_2, rt, 5 hours, 88%; 3) H_2O, reflux, 6 hours; 4) EtO–CH=N–CH(CN)CO_2Et, reflux, 15 minutes, 62% in 2 steps; 5) EtOCONCS, CH_3CN, reflux, 2 hours, 93%; 6) MeI, 0.1 N-NaOH, MeOH, rt, 1 hour, 99%; 7) 5 N-methanolic KOH, reflux, 30 minutes, then 4 N-HCl, rt, 10 minutes, 45%; 8) TFA : H_2O=3 : 1, 50°C, 3 hours, 99%; 9) $PO(OMe)_3$, $POCl_3$, 0°C, 2 hours, 44%.

Scheme 3.



Reagents and conditions: 1) 90% TFA, rt, 15 minutes; 2) EtO–CH=N–CH(CN)CO_2Et, reflux, 15 minutes, 79% in 2 steps; 3) EtOCONCS, CH_3CN, reflux, 1 hour, 88%; 4) MeI, 0.1 N-NaOH, MeOH, rt, 1 hour, 92%; 5) 5 N-methanolic KOH, reflux, 30 minutes, then 4 N-HCl, 56%; 6) H_2, 10% Pd/C, EtOH, rt, 14 hours, 92%.

Table 1. Inhibition of HIV-1 replication by oxanosine derivatives.

compound	CEM cells (acute[a])		H9 cells (acute[a])		U937 cells (chronic[b])	
	EC_{50}[c] (μg/ml)	CC_{50}[d] (μg/ml)	EC_{50}	CC_{50}	EC_{50}	CC_{50}
1	7.0	440	> 500	> 500	27	> 100
2	11	300	155	> 500	13	56
3	> 500	> 500	> 500	> 500	> 100	> 100
4	4.1	> 500	> 500	> 500	21	> 100
5	240	> 500	> 500	> 500	> 100	> 100
6	42	> 500	320	> 500	41	> 100
7	170	> 500	175	> 500	36	> 100
8	62	> 500	> 500	> 500	> 100	> 100
9	4.8	> 500	> 500	> 500	> 100	> 100
ddI	2.6	> 500	1.6	> 500	39	> 100
AZT	0.0018	> 500	0.052	> 500	9.4	> 100

[a]To evaluate anti-HIV activities toward acute infection, we used HIV-1 IIIB strain and CEM or H9 cells. The cells were pretreated with the compounds for 30 min and then infected by HIV at a multiplicity of 0.05. Cells were incubated for 90 min with virus and then diluted with fresh medium 1:10 for culturing. On day 6, the culture fluid was harvested for reverse transcriptase (RT) and MTT assays. [b]To evaluate anti-HIV activities toward chronic infection, we used HIV-1 IIIB strain and U937 cells. Chronically infected cells were seeded in the presence of the compounds and harvested at 96 hours for RT and MTT assays. [c]Effective concentration required to inhibit HIV-1 reverse transcriptase activity by 50 %. [d]50 % cytotoxic concentration.

500 μg/ml. These activities were comparable to that of ddI (EC_{50}, 2.6 μg/ml). Compound **4** was stronger than oxanosine **1**, and **8** was stronger than **5**. This may be because cellular phosphorylation is necessary for these unusual nucleosides to inhibit reverse transcriptase. Carbocyclic oxanosine **5** and its phosphate **8** were weaker than **1** and **4**, suggesting that the hydrofuran structure is essential. Oxanosine and related compounds showed only weak activity in H9 cells, although ddI exhibited anti-HIV activity with an EC_{50} value of 1.6 μg/ml in this cell line.

The chronic assay is considered to be closer to the clinical situation than the acute assay. Interestingly, oxanosine **1** and **4** also exhibited chronic anti-HIV-1 activity with EC_{50} values of 27 and 21 μg/ml, respectively, without cytotoxicity up to 100 μg/ml in U937 cells. Although **2** also showed activity (EC_{50}, 13 μg/ml), it exhibited cytotoxicity with a CC_{50} value of 56 μg/ml.

Thus, oxanosine and its derivatives showed anti-HIV activity in cellular acute and chronic assays. Oxanosine derivatives were more potent than the carbocyclic counterparts. The mechanism of anti-HIV effect is being studied.

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