Synthesis of sugar-modified derivatives of the unusual nucleoside oxanosine and its carbocyclic analogs as potential inhibitors of HIV [1]

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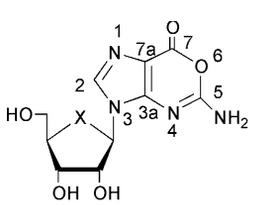
A series of sugar-modified derivatives of oxanosine and its carbocyclic analogs were synthesized from natural oxanosine and (−)-2-azabicyclo[2.2.1]hept-5-en-3-one, respectively. Among nucleosides tested for anti-HIV activities *in vitro*, oxanosine **1**, its 5′-monophosphate **9**, and 2′-deoxyoxanosine **8** reduced the number of HIV particles in CEM cells to almost the same level as ddI.

Introduction

Since the first description of AIDS (Acquired Immunodeficiency Syndrome) in 1981 and the discovery of the causative agent, the human immunodeficiency virus (HIV), there has been intense research aimed at identifying substances effective against HIV. The drugs currently approved for the clinical treatment of AIDS and AIDS-related complex are AZT, ddI, ddC, d4T, (−)-3TC and 1592U89 (avacavir™). However, the discovery of both clinical resistance and the expression of toxicity toward the used agents has emerged as a major concern with the effectiveness of long-term treatment. Thus, there is still a need for a potent and safer drug to treat HIV infections alone or in combination with other antiviral agents. 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.[2,3] Furthermore, **1** has been shown to alter tumor cell morphology into the normal morphology in temperature-sensitive K-ras transformed rat kidney (K-ras^{ts}-NRK) cells by inhibiting inosine monophosphate (IMP) dehydrogenase.[4]

However, the antiviral effect of oxanosine has not yet been investigated. In the search for effective, selective and nontoxic antiviral agents, a variety of strategies have been devised to design nucleoside analogs. These strategies have involved several formal modifications of the naturally occurring nucleosides, especially, alteration of the carbohydrate moiety. The sugar modifications make certain nucleosides acid stable and increase the metabolic stability by making them more resistant to hydrolysis by adenosine deaminase, as well as resistant to degradation by purine nucleoside phosphorylase. For a structure–activity relationship study, as well as for the purpose of finding new anti-HIV agents, we set ourselves toward the synthesis of a series of derivatives of oxanosine **1** and carbocyclic oxanosine **2** (Fig. 1) starting from natural oxanosine and (−)-2-azabicyclo[2.2.1]hept-5-en-3-one **10**, respectively.



1 X = O, oxanosine
2 X = CH_2, carbocyclic oxanosine

Fig. 1

Results and discussion

The synthesis of 2′,3′-didehydro-2′3′-dideoxyoxanosine **6**, 2′,3′-dideoxyoxanosine **7**, 2′-deoxyoxanosine **8** and oxanosine 5′-monophosphate **9** has been accomplished from natural oxanosine **1** as depicted in Scheme 1. After selective protection of the 5′-hydroxy group of **1** isolated from the culture broth of *Streptomyces capreolus* MG265-CF3 with *tert*-butyldimethylsilyl chloride (TBDMSCl) in pyridine at room temperature, treatment of silyl ether **3** with thiocarbonyldiimidazole (TCDI) in 1,2-dichloroethane afforded thiocarbonate **4** in 67% yield. Subsequently, compound **4** was then refluxed with trimethyl phosphite for 1.5 h to provide olefin **5** in 76% yield.[5] Deprotection of **5** with *n*-Bu_4NF (TBAF) in THF at room temperature gave 2′,3′-didehydro-2′,3′-dideoxyoxanosine **6** in 84% yield. Hydrogenation of **6** over 10% Pd/C in MeOH under hydrogen gas atmosphere afforded 2′,3′-dideoxyoxanosine **7** in 81% yield. 2′-Deoxyoxanosine **8** was obtained from **1** in 4 steps according to the known procedures.[6] Reaction of **1** with phosphoryl trichloride in trimethyl phosphate at 0 °C gave 5′-monophosphate **9** in 93% yield.[7]

The synthesis of optically active carbocyclic oxanosine **2**, 2′,3′-didehydro-2′,3′-dideoxycarbocyclic oxanosine **23** and 2′,3′-dideoxycarbocyclic oxanosine **24** has been accomplished according to Schemes 2 and 3. Fairly recently we reported the first synthesis of **2** from D-ribonic acid γ-lactone.[8] However, this route involved some troublesome steps, so a more convenient and versatile route was required. In the present paper we describe an alternative new route to **2** starting from com-

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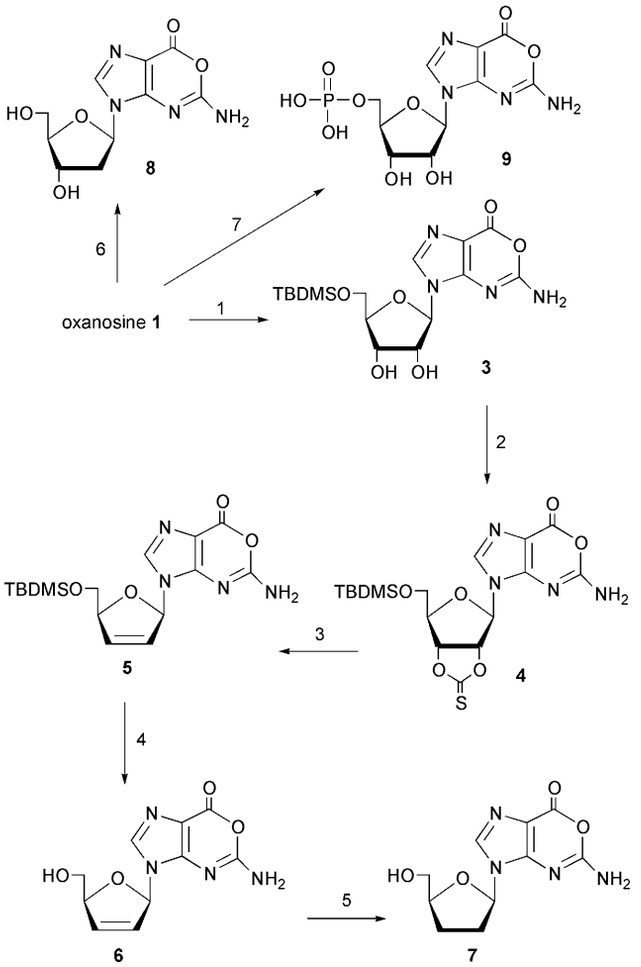
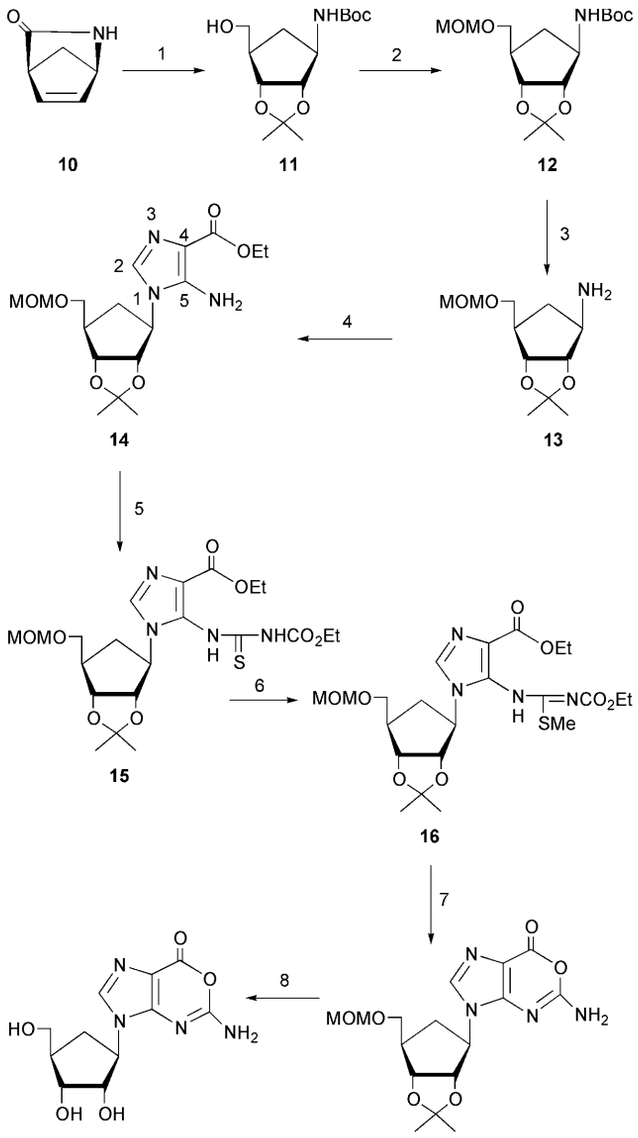


Scheme 1 *Reagents and conditions*: 1) TBDMSCl, pyridine, rt, 2 h; 2) TCDI, $ClCH_2CH_2Cl$, rt, 4 h; 3) $P(OMe)_3$, 120 °C, 1.5 h; 4) nBu_4NF, THF, rt, 1 h; 5) H_2, 10% Pd-C, MeOH, rt, 3 h; 6) 4 steps. See ref. 6(*a*); 7) $PO(OMe)_3$, $POCl_3$, 0 °C, 2 h.



Scheme 2 *Reagents and conditions*: 1) 4 steps. See ref. 9; 2) $ClCH_2OCH_3$, *N*,*N*-diisopropylethylamine, CH_2Cl_2, rt, 5 h; 3) water, reflux, 6 h; 4) EtO-CH=NCH(CN)CO_2Et, CH_3CN, reflux, 15 min; 5) EtOCONCS, CH_3CN, reflux, 2 h; 6) MeI, 0.1 M NaOH, MeOH, rt, 1 h; 7) 5 M methanolic KOH, reflux, 30 min; then 4 M HCl, rt, 10 min; 8) TFA–water 3 : 1, 50 °C, 3 h.

mercially available (−)-2-azabicyclo[2.2.1]hept-5-en-3-one **10**. At first, compound **10** was converted to *N*-Boc-alcohol **11** in four steps according to the protocol of Hutchinson *et al*.[9] Then, protection of the primary hydroxy group of **11** with chloromethyl methyl ether in the presence of *N*,*N*-diisopropylethylamine in dichloromethane gave methoxymethyl ether **12** in 88% yield. Deprotection of the *N*-Boc grouping of **12** under neutral conditions was achieved by refluxing with water for 6 h to afford amine **13**, which was, without purification, allowed to react with ethyl *N*-[ethoxycarbonyl(cyano)methyl]-formimidate[10] in refluxing CH_3CN to furnish the imidazole **14** in 62% yield in 2 steps. Then, reaction of **14** with ethoxycarbonyl isothiocyanate in refluxing CH_3CN gave the thiourea **15** in 93% yield, which on treatment with iodomethane in 0.1 M NaOH yielded the methylthio derivative **16** in 99% yield. Oxazinone ring formation from compound **16** was accomplished by reaction with 5 M methanolic KOH under reflux for 30 min followed by neutralization of the reaction mixture with 4 M HCl to afford imidazo-oxazinone **17** in 45% yield.[11] Carbocyclic oxanosine **2** could be prepared from compound **17** by a simultaneous deprotection of two kinds of protecting groups with aq. trifluoroacetic acid (TFA) in 99% yield.

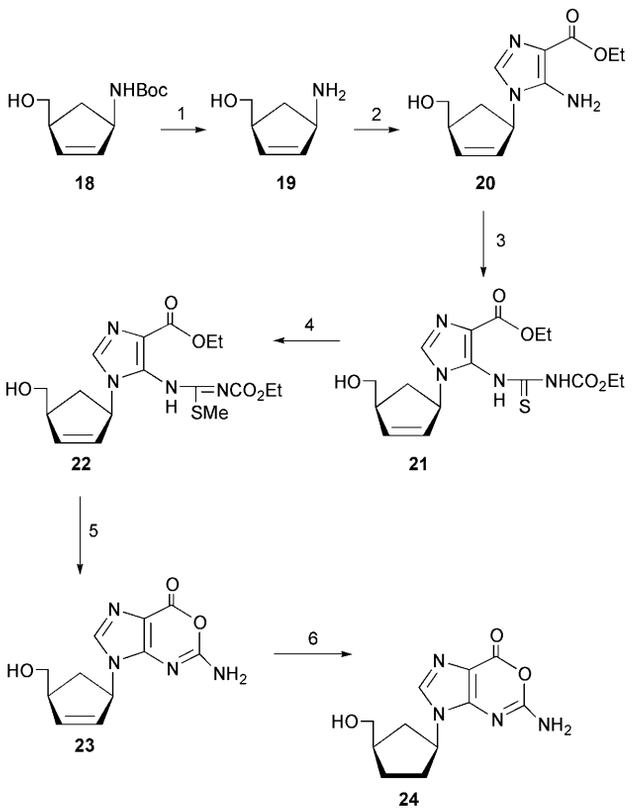
By employing similar reactions to those described for **2** from **13**, we obtained carbovir-type oxanosine analog **23** from compound **18** in 5 steps. Deprotection of the *N*-Boc grouping of compound **18** prepared from **10** in two steps[9] with 90% TFA afforded amino alcohol **19**, which reacted directly with ethyl *N*-[ethoxycarbonyl(cyano)methyl]formimidate in refluxing CH_3CN to furnish imidazole **20** in 79% yield in 2 steps. Then, reaction of **20** with ethoxycarbonyl isothiocyanate in refluxing CH_3CN provided the thiourea **21** in 88% yield. Treatment of **21** with iodomethane in 0.1 M NaOH followed by cyclization reaction with 5 M methanolic KOH and subsequent acidification with 4 M HCl *via* compound **22** provided 2′,3′-didehydro-2′,3′-dideoxycarbocyclic oxanosine **23** in 52% overall yield. Hydrogenation of **23** over 10% Pd/C in EtOH under hydrogen atmosphere afforded carbocyclic 2′,3′-dideoxyoxanosine **24** in 92% yield.

In conclusion, we have successfully synthesized a series of sugar-modified derivatives of oxanosine and its carbocyclic analogs for evaluation of their HIV-1 activity. Also, a simple and high-yield synthetic pathway has been developed for the preparation of carbocyclic oxanosine.

Experimental

General

^1H NMR and ^{13}C NMR spectra were recorded with JEOL JNM Lambda-300, JNM GX-400 or JNM ALPHA-400 spectrometers. Chemical shifts (δ) are expressed in ppm from



Scheme 3 *Reagents and conditions*: 1) 90% TFA, rt, 15 min; 2) EtO-CH=NCH(CN)CO_2Et, CH_3CN, reflux, 15 min; 3) EtOCONCS, CH_3CN, reflux, 1 h; 4) MeI, 0.1 M NaOH, MeOH, rt, 1 h; 5) 5 M methanolic KOH, reflux, 30 min; then 4 M HCl; 6) H_2, 10% Pd/C, EtOH, rt, 14 h.

Me_4Si as an internal standard. UV spectra were recorded on a JASCO UVIDEC-610 spectrophotometer. Mass spectra were recorded in the EI mode with a Hitachi M-80 or JEOL JMS-GCMATE mass spectrometer at an ionization energy of 70 eV. IR spectra were recorded on a Bio-Rad FTS-165 or FTS-65 infrared spectrophotometer. Optical rotations were recorded at the sodium D-line and at ambient temperature with a JASCO DIP-360. $[a]_D$-Values are given in units of 10^{-1} deg cm^2 g^{-1}. Mps were measured on Yanaco MP-S3 and are uncorrected. Only the strongest and/or structurally important peaks are reported for the IR spectra. Silica gel column chromatography was carried out using Silica Gel 60 (E. Merck, Darmstadt). TLC was carried out on 0.25 mm precoated plates of Silica Gel 60 F_{254} (E. Merck, Darmstadt). Reaction progress was monitored by either UV (254 nm) or staining with 5% phosphomolybdic acid in ethanol as developing agent, followed in the latter case by heating on an electric plate. Preparative-layer chromatography (PLC) was performed on 0.25, 0.5, and 1 mm × 20 cm × 20 cm precoated silica gel-60 (60F-254) (E. Merck, Darmstadt) plates. Dichloromethane and acetonitrile were distilled over calcium hydride. Methanol and ethanol were distilled over magnesium. THF was distilled over sodium benzophenone ketyl prior to use. Reactions were carried out under an argon atmosphere unless otherwise stated. All evaporations were carried out on a rotary evaporator under reduced pressure. Reaction temperatures were measured externally. Yields refer to chromatographically and spectroscopically pure compounds.

5′-*O*-(*tert*-Butyldimethylsilyl)oxanosine 3

A solution of natural oxanosine **1** (97.4 mg, 0.343 mmol), obtained by the fermentation of *S. capreolus* MG265-CF3, and TBDMSCl (54.3 mg, 0.360 mmol) in dry pyridine (3.0 cm^3) was stirred at RT for 2 h. After concentration of the solution to dryness, the residue was purified by silica gel column chromatography ($CHCl_3$–MeOH 7:1) to give *title compound* **3** (123 mg, 90%) as a colorless powder, mp 176–178 °C; ν_{max} (KBr)/cm^{-1} 3359, 1772, 1654, 1553, 1107 and 1082; $[a]_D^{20}$ −15 (*c* 1.0 in MeOH); δ_H (CD_3OD) 0.108 (3H, s, CH_3), 0.113 (3H, s, CH_3), 0.926 (9H, s, Bu^t), 3.84 (1H, dd, *J* 2.9 and 11.7 Hz, 5′-H^a), 3.95 (1H, dd, *J* 2.9 and 11.7 Hz, 5′-H^b), 4.07 (1H, ddd, *J* 2.9, 2.9 and 4.8 Hz, 4′-H), 4.23 (1H, dd, *J* 4.8 and 5.1 Hz, 3′-H), 4.29 (1H, dd, *J* 4.8 and 5.1 Hz, 2′-H), 5.81 (1H, d, *J* 4.8 Hz, 1′-H) and 7.96 (1H, s, 2-H); δ_C (CD_3OD; 75.5 MHz) −5.35 ($SiCH_3$), −5.30 ($SiCH_3$), 19.3 [$C(CH_3)_3$], 26.5 (CH_3 × 3), 64.0 (C-5′), 71.5 (C-3′), 76.5 (C-2′), 86.5 (C-4′), 89.5 (C-1′), 112.5 (C-7a), 137.9 (C-2), 154.4 (C-3a), 155.9 (C-5) and 161.9 (C-7); *m*/*z* (EI) 383.1361. $C_{15}H_{23}N_4O_6Si$ (M^+ − Me) requires *m*/*z*, 383.1386 (Found: C, 48.42; H, 6.49; N, 14.21. $C_{16}H_{26}N_4O_6Si$ requires C, 48.23; H, 6.58; N, 14.06%).

5′-*O*-(*tert*-Butyldimethylsilyl)oxanosine 2′,3′-thiocarbonate 4

A mixture of silyl ether **3** (116 mg, 0.291 mmol) and TCDI (103.6 mg, 0.582 mmol) in 1,2-dichloroethane (9.0 cm^3) was stirred at RT for 4 h. After evaporation of the solvent, the residue was purified by silica gel column chromatography ($CHCl_3$–MeOH 10:1) to give *title compound* **4** (85.3 mg, 67%) as a colorless powder, mp 171–174 °C; ν_{max} (KBr)/cm^{-1} 3422, 1771, 1648, 1638, 1558, 1146 and 1104; $[a]_D^{21}$ −22 (*c* 0.53 in MeOH); δ_H (CD_3OD) −0.017 (3H, s, CH_3), −0.005 (3H, s, CH_3), 0.832 (9H, s, Bu^t), 3.88 (1H, dd, *J* 5.1 and 11.0 Hz, 5′-H^a), 3.99 (1H, dd, *J* 5.1 and 11.0 Hz, 5′-H^b), 4.55 (1H, dt, *J* 2.6 and 5.1 Hz, 4′-H), 5.75 (1H, dd, *J* 2.6 and 7.0 Hz, 3′-H), 6.17 (1H, dd, *J* 1.5 and 7.0 Hz, 2′-H), 6.29 (1H, d, *J* 1.5 Hz, 1′-H) and 7.86 (1H, s, 2-H); δ_C (CD_3OD; 75.5 MHz) −5.51 ($SiCH_3$), −5.45 ($SiCH_3$), 19.1 [$C(CH_3)_3$], 26.3 (CH_3 × 3), 64.2 (C-5′), 87.8 (C-4′), 89.1 (C-1′), 89.6 (C-3′), 91.3 (C-2′), 112.8 (C-7a), 139.0 (C-2), 153.5 (C-3a), 155.5 (C-5), 161.9 (C-7) and 191.6 (C=S); *m*/*z* (EI) 383.0481. $C_{13}H_{15}N_4O_6SSi$ (M^+ − ^{t}Bu) requires *m*/*z*, 383.0484 (Found: C, 46.55; H, 5.37; N, 12.91. $C_{17}H_{24}N_4O_6SSi$ requires C, 46.35; H, 5.49; N, 12.72%).

5′-*O*-(*tert*-Butyldimethylsilyl)-2′,3′-didehydro-2′,3′-dideoxoxanosine 5

A solution of thiocarbonate **4** (13.0 mg, 0.0296 mmol) in trimethyl phosphite (1.5 cm^3) was refluxed for 1.5 h. After completion of the reaction, the excess of trimethyl phosphite was removed *in vacuo*. The resulting residue was purified by silica gel column chromatography ($CHCl_3$–MeOH 10:1) to give *title compound* **5** (8.2 mg, 76%) as a colorless powder, mp 103–111 °C; ν_{max} (KBr)/cm^{-1} 3410, 1758, 1684 and 1560; $[a]_D^{20}$ −47 (*c* 0.40 in MeOH); δ_H ($CDCl_3$) 0.040 (6H, s, 2 × CH_3), 0.88 (9H, s, Bu^t), 3.74 (1H, dd, *J* 4.8 and 11.0 Hz, 5′-H^a), 3.81 (1H, dd, *J* 3.7 and 11.0 Hz, 5′-H^b), 4.96 (1H, m, 4′-H), 5.98 (1H, ddd, *J* 1.5, 1.8 and 6.2 Hz, 3′-H), 6.43 (1H, ddd, *J* 1.5, 1.8 and 6.2 Hz, 2′-H), 6.75 (1H, ddd, *J* 1.5, 1.5 and 2.9 Hz, 1′-H) and 7.72 (1H, s, 2-H); δ_C (CD_3OD; 75.5 MHz) −5.25 [$Si(CH_3)_2$], 19.5 [$C(CH_3)_3$], 26.5 (CH_3 × 3), 66.0 (C-5′), 89.6 (C-4′), 89.7 (C-1′), 112.8 (C-7a), 126.5 (C-3′), 135.6 (C-2′), 138.4 (C-2), 154.3 (C-3a), 156.0 (C-5) and 162.0 (C-7); *m*/*z* (EI) 307.0867. $C_{12}H_{15}N_4O_4Si$ (M^+ − ^{t}Bu) requires *m*/*z*, 307.0862 (Found: C, 52.48; H, 6.81; N, 15.59. $C_{16}H_{24}N_4O_4Si$ requires C, 52.73; H, 6.64; N, 15.37%).

2′,3′-Didehydro-2′,3′-dideoxyoxanosine 6

To a solution of olefin **5** (38.4 mg, 1.05 mmol) in dry THF (8.0 cm^3) was added TBAF (1.0 M in THF; 2.1 cm^3) at 0 °C and the reaction mixture was stirred at RT for 1 h before being concentrated to dryness, and the residue was purified by silica gel column chromatography ($CHCl_3$–MeOH 10:1) to give *title compound* **6** (22.0 mg, 84%) as a colorless powder, mp 119–126 °C; ν_{max} (KBr)/cm^{-1} 3428, 1794, 1654 and 1560; $[a]_D^{21}$ −49 (*c* 0.50 in MeOH); δ_H (CD_3OD) 3.70–3.72 (2H, m, 5′-H_2), 4.63

(1H, m, 4′-H), 6.06 (1H, ddd, *J* 1.5, 2.2 and 5.9 Hz, 3′-H), 6.43 (1H, ddd, *J* 1.5, 1.8 and 5.9 Hz, 2′-H), 6.79 (1H, ddd, *J* 1.5, 1.5 and 1.8 Hz, 1′-H) and 7.90 (s, 1H, 2-H); δ_C (CD_3OD; 75.5 MHz) 64.1 (C-5′), 89.7 (C-4′), 89.8 (C-1′), 112.2 (C-7a), 126.6 (C-3′), 135.6 (C-2′), 138.7 (C-2), 154.3 (C-3a), 156.0 (C-5) and 161.9 (C-7); *m*/*z* (EI) 250.0707. $C_{10}H_{10}N_4O_4$ requires *M*, 250.0702 (Found: C, 47.79; H, 3.86; N, 22.52. $C_{10}H_{10}N_4O_4$ requires C, 48.00; H, 4.03; N, 22.39%).

2′,3′-Dideoxyoxanosine 7

A mixture of compound **6** (7.1 mg, 0.0248 mmol) and 10% Pd/C (0.5 mg) in MeOH (1.5 cm^3) was stirred under hydrogen atmosphere for 3 h. The reaction mixture was filtered through a Celite pad and washed with MeOH (12 cm^3), the filtrate and washings being combined. After evaporation of the solvent, the residue was purified by PLC ($CHCl_3$–MeOH 6:1) to give *title compound* **7** (5.8 mg, 81%) as a colorless powder, mp 103–112 °C; ν_{max} (KBr)/cm^{-1} 3417, 1772, 1654 and 1559; $[\alpha]_D^{18}$ −1.1 (*c* 0.1 in MeOH); δ_H (CD_3OD) 2.02–2.15 (2H, m, 3′-H_2), 2.36–2.50 (2H, m, 2′-H_2), 3.65 (1H, dd, *J* 4.4 and 12.1 Hz, 5′-H^a), 3.81 (1H, dd, *J* 3.3 and 12.1 Hz, 5′-H^b), 4.19 (1H, m, 4′-H), 6.06 (1H, dd, *J* 3.7 and 6.6 Hz, 1′-H) and 8.04 (1H, s, 2-H); δ_C (CD_3OD; 100.5 MHz) 26.5 (C-3′), 33.6 (C-2′), 64.3 (C-5′), 83.6 (C-4′), 86.6 (C-1′), 112.8 (C-7a), 138.2 (C-2), 153.2 (C-3a), 157.6 (C-5) and 161.5 (C-7); *m*/*z* (EI) 252.0854. $C_{10}H_{12}N_4O_4$ requires *M*, 252.0858 (Found: C, 47.75; H, 4.68; N, 22.47. $C_{10}H_{12}N_4O_4$ requires C, 47.62; H, 4.80; N, 22.21%).

Oxanosine 5′-monophosphate 9

To a solution of oxanosine **1** (10 mg, 0.035 mmol) in trimethyl phosphate (0.7 cm^3) was added phosphoryl trichloride (0.1 cm^3) at 0 °C and the reaction mixture was stirred at 0 °C for 2 h before being diluted with water (10 cm^3) and washed with Et_2O (20 cm^3). The water layers were concentrated, and purified by Toyopearl HW-40 column chromatography (MeOH) to afford *title compound* **9** (11.9 mg, 93%) as a colorless, waxy solid, ν_{max} (KBr)/cm^{-1} 3423, 1794, 1655, 1578, 1104 and 1058; $[\alpha]_D^{18}$ −27 (*c* 0.45 in MeOH); δ_H (CD_3OD) 4.13–4.25 (3H, m, 4′-H, 5′-H_2), 4.32 (1H, dd, *J* 3.3 and 5.1 Hz, 3′-H), 4.57 (1H, dd, *J* 5.1 and 5.5 Hz, 2′-H), 5.83 (1H, d, *J* 5.5 Hz, 1′-H) and 8.03 (1H, s, 2-H); δ_C (CD_3OD; 75.5 MHz) 66.8 (C-5′), 71.9 (C-3′), 75.6 (C-2′), 85.0 (C-4′), 89.7 (C-1′), 112.1 (C-7a), 138.4 (C-2), 154.5 (C-3a), 155.6 (C-5) and 161.9 (C-7); *m*/*z* (FAB) 364.0400. $C_{10}H_{13}N_4O_9P$ requires *M*, 364.0420.

(1*R*,2*S*,3*R*,4*R*)-1-(*tert*-Butoxycarbonylamino)-2,3-isopropylidenedioxy-4-(methoxymethoxymethyl)cyclopentane 12

To a solution of the alcohol **11** (106.8 mg, 0.372 mmol) in CH_2Cl_2 (2.0 cm^3) were added chloromethyl methyl ether (0.10 cm^3, 1.34 mmol) and *N*,*N*-diisopropylethylamine (0.16 cm^3, 0.893 mmol). The reaction mixture was stirred at RT for 5 h. After concentration of the solution to dryness, the residue was extracted with EtOAc (10 cm^3 × 3). The organic layer was washed with brine (10 cm^3 × 3), dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by PTLC ($CHCl_3$–MeOH 10:1) to afford *title compound* **12** (108.8 mg, 88%) as a colorless, waxy solid, ν_{max} (film)/cm^{-1} 1716, 1523 and 1163; $[\alpha]_D^{23}$ −2.4 (*c* 1.0 in $CHCl_3$); δ_H ($CDCl_3$) 1.29 (3H, s, CH_3), 1.44 (9H, s, Bu^t), 1.47 (3H, s, CH_3), 2.33–2.38 (2H, m, 5-H_2), 2.48 (1H, m, 4-H), 3.39 (3H, s, OCH_3), 3.56 (1H, dd, *J* 4.8 and 9.9 Hz, 6-H^a), 3.62 (1H, dd, *J* 4.8 and 9.9 Hz, 6-H^b), 4.03 (1H, m, 1-H), 4.38 (1H, dd, *J* 2.6 and 5.9 Hz, 3-H), 4.50 (1H, dd, *J* 2.6 and 5.9 Hz, 2-H), 4.66 (2H, s, OCH_2O) and 5.41 (1H, br s, NH); δ_C ($CDCl_3$; 75.5 MHz) 24.7 [C(CH_3)$_2$], 27.1 [C(CH_3)$_2$], 33.8 (C-5), 45.1 (C-4), 55.6 (OCH_3), 56.6 (C-1), 69.4 (CH_2OMOM), 79.1 [OC(CH_3)$_3$], 83.3 (C-3), 86.9 (C-2), 96.6 (OCH_2OCH$_3$), 111.2 [OC(CH_3)$_2$O] and 155.2 (NHCOO); *m*/*z* (EI) 332.2059. $C_{16}H_{30}NO_6$ (M^+ + H) requires *m*/*z*, 332.2073.

Ethyl (1′*R*,2′*S*,3′*R*,4′*R*)-5-amino-1-[2′,3′-isopropylidenedioxy-4′-(methoxymethoxymethyl)cyclopentanyl]-1*H*-imidazole-4-carboxylate 14

A solution of methoxymethyl ether **12** (461.7 mg, 1.40 mmol) in water (10 cm^3) was heated at 110 °C for 6 h. After completion of the reaction, the solvent was removed *in vacuo* to afford crude deprotected amine **13**, which was used in the next reaction without purification.

To a solution of ethyl *N*-[ethoxycarbonyl(cyano)methyl]-formimidate (400 mg) in CH_3CN (5 cm^3) was added a solution of crude amine **13** in CH_3CN (3 cm^3) and the mixture was refluxed at 90 °C for 15 min. The reaction mixture was concentrated to dryness, and the residue was purified by silica gel column chromatography (*n*-hexane–EtOAc 1:2) to give *title compound* **14** (320.6 mg, 62%) as a colorless powder, mp 51–55 °C; ν_{max} (film)/cm^{-1} 1672, 1625, 1561, 1212, 1125 and 1032; $[\alpha]_D^{26}$ −6.4 (*c* 1.0 in $CHCl_3$); δ_H ($CDCl_3$) 1.29 (3H, s, CH_3), 1.34 (3H, t, *J* 6.8 Hz, CH_2CH_3), 1.57 (3H, s, CH_3), 2.20 (1H, ddd, *J* 12.2, 12.2 and 12.2 Hz, 5′-H^a), 2.39–2.48 (2H, m, 4′-H and 5′-H^b), 3.35 (3H, s, OCH_3), 3.60 (1H, dd, *J* 5.4 and 9.8 Hz, 6′-H^a), 3.67 (1H, dd, *J* 4.9 and 9.8 Hz, 6′-H^b), 4.16 (1H, ddd, *J* 6.4, 6.4 and 12.2 Hz, 1′-H), 4.29 (2H, q, *J* 6.8 Hz, CH_2CH_3), 4.39 (1H, dd, *J* 4.5 and 6.4 Hz, 3′-H), 4.55 (1H, dd, *J* 4.5 and 6.4 Hz, 2′-H), 4.63 (2H, s, OCH_2O), 5.54 (2H, br s, NH_2) and 6.94 (1H, s, 2-H); δ_C ($CDCl_3$; 100.5 MHz) 14.7 ($CO_2CH_2CH_3$), 25.0 [C(CH_3)$_2$], 27.3 [C(CH_3)$_2$], 31.6 (C-5′), 43.2 (C-4′), 55.5 (OCH_3), 59.7 (C-1′), 60.8 ($CO_2CH_2CH_3$), 67.9 (C-6′), 81.4 (C-3′), 85.4 (C-2′), 96.6 (OCH_2OCH$_3$), 111.6 [OC(CH_3)$_2$O], 114.5 (C-4), 127.4 (C-2), 146.2 (C-5) and 164.6 ($CO_2CH_2CH_3$); *m*/*z* (EI) 369.1881. $C_{17}H_{27}N_3O_6$ requires *M*, 369.1899 (Found: C, 55.53; H, 7.51; N, 11.19. $C_{17}H_{27}N_3O_6$ requires C, 55.27; H, 7.37; N, 11.37%).

Ethyl (1′*R*,2′*S*,3′*R*,4′*R*)-5-[*N*′-(ethoxycarbonyl)thioureido]-1-[2′,3′-isopropylidenedioxy-4′-(methoxymethoxymethyl)cyclopentanyl]-1*H*-imidazole-4-carboxylate 15

A solution of ester **14** (132.9 mg, 0.360 mmol) and ethoxycarbonyl isothiocyanate (66 mg, 0.5 mmol) in CH_3CN (4.0 cm^3) was refluxed for 2 h. The reaction mixture was concentrated to dryness and the residue was purified by silica gel column chromatography ($CHCl_3$–MeOH 10:1) to give *title compound* **15** (167.0 mg, 93%) as a colorless foam, ν_{max} (film)/cm^{-1} 1716, 1507, 1158 and 1041; $[\alpha]_D^{21}$ −36 (*c* 1.0 in $CHCl_3$); δ_H ($CDCl_3$) 1.24 (3H, s, CH_3), 1.35 (6H, t, *J* 7.3 Hz, 2 × CH_2CH_3), 1.49 (3H, s, CH_3), 2.10 (1H, m, 5′-H^a), 2.45 (1H, m, 5′-H^b), 2.59 (1H, m, 4′-H), 3.38 (3H, s, OCH_3), 3.61 (1H, dd, *J* 5.4 and 9.8 Hz, 6′-H^a), 3.69 (1H, dd, *J* 4.9 and 9.8 Hz, 6′-H^b), 4.31 (2H, q, *J* 7.3 Hz, CH_2CH_3), 4.35 (2H, q, *J* 7.3 Hz, CH_2CH_3), 4.50–4.58 (3H, m, 1′-, 2′- and 3′-H), 4.67 (2H, s, OCH_2O), 7.61 (1H, s, 2-H), 8.43 (1H, br s, NH) and 11.1 (1H, br s, NH); δ_C ($CDCl_3$; 75.5 MHz) 14.1 ($NHCO_2CH_2CH_3$), 14.2 ($CO_2CH_2CH_3$), 25.1 [C(CH_3)$_2$], 27.3 [C(CH_3)$_2$], 32.9 (C-5′), 43.0 (C-4′), 55.4 (OCH_3), 60.6 (C-1′), 61.3 ($NHCO_2CH_2CH_3$), 63.3 ($CO_2CH_2CH_3$), 67.8 (C-6′), 81.2 (C-3′), 85.6 (C-2′), 96.5 (OCH_2OCH$_3$), 113.6 [OC(CH_3)$_2$O], 126.6 (C-4), 131.5 (C-2), 133.0 (C-5), 152.5 ($NHCO_2CH_2CH_3$), 162.0 ($CO_2CH_2CH_3$) and 181.6 (NHCSNH); *m*/*z* (EI) 500.1296. $C_{21}H_{32}N_4O_8S$ requires *M*, 500.1940.

Ethyl (1′*R*,2′*S*,3′*R*,4′*R*)-5-(*N*′-ethoxycarbonyl-*S*-methylisothioureido)-1-[2′,3′-isopropylidenedioxy-4′-(methoxymethoxymethyl)cyclopentanyl]-1*H*-imidazole-4-carboxylate 16

To a solution of the thiourea **15** (519.4 mg, 1.04 mmol) in a mixture of MeOH (10 cm^3) and 0.1 M NaOH (10 cm^3) was added iodomethane (0.13 cm^3, 2.08 mmol). The reaction

mixture was stirred at RT for 1 h. After concentration of the solution to dryness, the residue was purified by silica gel column chromatography ($CHCl_3$–MeOH 40:1) to give *title compound* **16** (529.6 mg, 99%) as a colorless, waxy solid, ν_{max} (film)/cm^{-1} 1742, 1707, 1609, 1216, 1070 and 1037; $[\alpha]_D^{22}$ −19 (*c* 1.0 in $CHCl_3$); δ_H ($CDCl_3$) 1.26 (3H, t, *J* 7.3 Hz, CH_2CH_3), 1.28 (3H, s, CH_3), 1.35 (3H, t, *J* 7.3 Hz, CH_2CH_3), 1.51 (3H, s, CH_3), 2.11 (1H, ddd, *J* 11.4, 11.4 and 11.4 Hz, 5′-H^a), 2.32–2.48 (2H, m, 4′-H and 5′-H^b), 2.49 (3H, s, SCH_3), 3.37 (3H, s, OCH_3), 3.60 (1H, dd, *J* 4.8 and 9.9 Hz, 6′-H^a), 3.66 (1H, dd, *J* 4.8 and 9.9 Hz, 6′-H^b), 4.17 (2H, q, *J* 7.3 Hz, CH_2CH_3), 4.33 (2H, q, *J* 7.3 Hz, CH_2CH_3), 4.42–4.64 (3H, m, 1′-, 2′- and 3′-H), 4.66 (2H, s, OCH_2O), 7.51 (1H, s, 2-H) and 7.77 (1H, br s, NH); δ_C ($CDCl_3$; 100.5 MHz) 14.2 (SCH_3), 14.4 ($NHCO_2CH_2CH_3$), 14.6 ($CO_2CH_2CH_3$), 25.1 [$C(CH_3)_2$], 27.5 [$C(CH_3)_2$], 33.5 (C-5′), 43.5 (C-4′), 55.4 (OCH_3), 60.4 (C-1′), 60.8 ($NHCO_2CH_2CH_3$), 62.5 ($CO_2CH_2CH_3$), 67.8 (C-6′), 81.2 (C-3′), 84.7 (C-2′), 96.5 (OCH_2OCH_3), 113.5 [$OC(CH_3)_2O$], 118.1 (C-4), 132.7 (C-2), 140.5 (C-5), 151.4 ($NHCO_2CH_2CH_3$), 159.0 [$NHC(SCH_3)$=N] and 162.8 ($CO_2CH_2CH_3$); *m*/*z* (EI) 514.2081. $C_{22}H_{34}N_4O_8S$ requires *M*, 514.2097.

(1′*R*,2′*S*,3′*R*,4′*R*)-5-Amino-3-[2′,3′-isopropylidenedioxy-4′-(methoxymethoxymethyl)cyclopentanyl]imidazo[4,5-*d*][1,3]-oxazin-7(3*H*)-one 17

A solution of sulfide **16** (135.3 mg, 0.263 mmol) and 5 M methanolic KOH (3 cm^3) was refluxed at 90 °C for 30 min. The reaction solution was then adjusted to pH 3 with 4 M hydrochloric acid at 0 °C. The reaction mixture was extracted with $CHCl_3$ (10 cm^3 × 2) and the combined organic layers were washed successively with water (10 cm^3) and brine (10 cm^3). The organic solution was dried over anhydrous Na_2SO_4, filtered, concentrated, and purified by silica gel column chromatography ($CHCl_3$–MeOH 20:1) to afford *title compound* **17** (43.4 mg, 45%) as a colorless, waxy solid, ν_{max} (film)/cm^{-1} 1772, 1647, 1557 and 1072; $[\alpha]_D^{21}$ −38 (*c* 1.0 in $CHCl_3$); δ_H ($CDCl_3$) 1.33 (3H, s, CH_3), 1.57 (3H, s, CH_3), 2.19 (1H, m, 5′-H^a), 2.42–2.50 (2H, m, 4′-H and 5′-H^b), 3.39 (3H, s, OCH_3), 3.67 (2H, d, *J* 5.4 Hz, 6′-H_2), 4.56–4.63 (2H, m, 2′- and 3′-H), 4.68 (2H, s, OCH_2O), 4.87 (1H, m, 1′-H), 5.41 (2H, br s, NH_2) and 7.58 (1H, s, 2-H); δ_C ($CDCl_3$; 75.5 MHz) 25.1 [$C(CH_3)_2$], 27.5 [$C(CH_3)_2$], 34.1 (C-5′), 43.7 (C-4′), 55.4 (OCH_3), 61.2 (C-1′), 68.3 (C-6′), 81.5 (C-3′), 83.9 (C-2′), 96.6 (OCH_2OCH_3), 113.1 [$OC(CH_3)_2O$], 113.8 (C-7a), 137.2 (C-2), 152.2 (C-3a), 154.2 (C-5) and 158.8 (C-7); *m*/*z* (EI) 366.1552, $C_{16}H_{22}N_4O_6$ requires *M*, 366.1539.

(1′*R*,2′*S*,3′*R*,4′*R*)-5-Amino-3-[2′,3′-dihydroxy-4′-(hydroxymethyl)cyclopentanyl]imidazo[4,5-*d*][1,3]oxazin-7(3*H*)-one (carbocyclic oxanosine) 2

A solution of compound **17** (21.8 mg, 0.0596 mmol) and TFA–water (3:1; 3 cm^3) was heated at 50 °C for 3 h. The reaction solution was evaporated to dryness and the residue was purified by HPLC (Pegasil ODS 20 × 250 mm, MeOH–water 2:3, flow rate 3 cm^3 min^{-1}; retention time 8.8 min) to afford *title compound* **2** (16.7 mg, 99%) as a colorless foam, ν_{max} (KBr)/cm^{-1} 3427, 1775, 1651, 1563 and 1115; λ_{max} (H_2O) 247 (ε 9770) and 288 nm (ε 7460); λ_{max} (0.1 M HCl) 248 (ε 9640) and 288 nm (ε 7190); λ_{max} (0.1 M NaOH) 273 nm (ε 7520); $[\alpha]_D^{21}$ −20 (*c* 1.16 in H_2O); δ_H (D_2O) 1.89 (1H, ddd, *J* 8.7, 10.4 and 13.0 Hz, 5′-H^a), 2.38 (1H, m, 4′-H), 2.57 (1H, dt, *J* 8.4 and 13.0 Hz, 5′-H^b), 3.84 (2H, d, *J* 6.3 Hz, 6′-H_2), 4.19 (1H, dd, *J* 3.5 and 5.7 Hz, 3′-H), 4.53 (1H, dd, *J* 5.7 and 9.1 Hz, 2′-H), 4.76 (1H, ddd, *J* 8.4, 9.1 and 10.4 Hz, 1′-H) and 8.03 (1H, s, 2-H); δ_C (D_2O; 100.5 MHz) 29.4 (C-5′), 45.6 (C-4′), 60.1 (C-1′), 63.9 (C-6′), 72.7 (C-3′), 76.1 (C-2′), 112.3 (C-7a), 139.7 (C-2), 154.4 (C-3a), 157.5 (C-5) and 160.5 (C-7); *m*/*z* (EI) 266.0733. $C_{11}H_{12}N_3O_5$ ($M^+ - NH_2$) requires *m*/*z*, 266.0776.

Ethyl (1′*R*,4′*S*)-5-amino-1-[4′-(hydroxymethyl)cyclopent-2′-enyl]-1*H*-imidazole-4-carboxylate 20

A solution of compound **18** (161 mg, 0.756 mmol) in 90% TFA (2.0 cm^3) was stirred at RT for 15 min. After completion of the reaction, the solvent was removed *in vacuo* to afford amine **19** (223.3 mg). Then, to the solution of the crude amine **19** (223 mg) in dry CH_3CN (2 cm^3) was added a solution of ethyl *N*-[ethoxycarbonyl(cyano)methyl]formimidate (265 mg, 1.44 mmol) in CH_3CN (3.8 cm^3) and the mixture was refluxed at 90 °C for 15 min. The reaction mixture was concentrated to dryness, and the residue was purified by silica gel column chromatography ($CHCl_3$–MeOH 7:1) to give *title compound* **20** (150.6 mg, 79% in 2 steps) as a colorless oil, ν_{max} (film)/cm^{-1} 3319, 1672, 1632, 1559, 1128 and 1033; $[\alpha]_D^{20}$ −34 (*c* 1.0 in MeOH); δ_H ($CDCl_3$) 1.37 (3H, t, *J* 7.3 Hz, CH_2CH_3), 1.91 (1H, ddd, *J* 6.6, 7.3 and 13.9 Hz, 5′-H^a), 2.65 (1H, ddd, *J* 5.1, 8.8 and 13.9 Hz, 5′-H^b), 3.03 (1H, m, 4′-H), 3.70 (1H, dd, *J* 4.4 and 10.6 Hz, 6′-H^a), 3.84 (1H, dd, *J* 4.4 and 10.6 Hz, 6′-H^b), 4.32 (2H, q, *J* 7.3 Hz, CH_2CH_3), 5.14 (1H, m, 1′-H), 5.31 (2H, br s, NH_2), 5.85 (1H, ddd, *J* 2.2, 2.5 and 5.9 Hz, 3′-H), 6.14 (1H, ddd, *J* 1.8, 2.2 and 5.9 Hz, 2′-H) and 7.02 (1H, s, 2-H); δ_C ($CDCl_3$; 75.5 MHz) 14.5 ($CO_2CH_2CH_3$), 31.9 (C-5′), 47.4 (C-4′), 59.6 ($CO_2CH_2CH_3$), 61.3 (C-1′), 63.8 (C-6′), 111.3 (C-4), 129.1 (C-3′), 130.6 (C-2), 138.9 (C-2′), 145.4 (C-5) and 164.7 ($CO_2CH_2CH_3$); *m*/*z* (EI) 251.1295, $C_{12}H_{17}N_3O_3$ requires *M*, 251.1270.

Ethyl (1′*R*,4′*S*)-5-[*N*′-(ethoxycarbonyl)ureido]-1-[4′-(hydroxymethyl)cyclopent-2′-enyl]-1*H*-imidazole-4-carboxylate 21

Compound **21** (83.2 mg, 88%) was prepared from **20** (62.1 mg, 0.247 mmol) by the method described for **15**. Purification by silica gel column chromatography ($CHCl_3$–MeOH 15:1) afforded *title compound* **21** as a colorless, waxy solid, ν_{max} (film)/cm^{-1} 3188, 1717, 1543, 1215 and 1177; $[\alpha]_D^{19}$ −23 (*c* 1.0 in MeOH); δ_H ($CDCl_3$) 1.35 (3H, t, *J* 7.0 Hz, CH_2CH_3), 1.36 (3H, t, *J* 7.0 Hz, CH_2CH_3), 1.76 (1H, ddd, *J* 5.9, 6.2 and 13.9 Hz, 5′-H^a), 2.77 (1H, ddd, *J* 8.8, 9.2 and 13.9 Hz, 5′-H^b), 2.99 (1H, m, 4′-H), 3.67 (1H, dd, *J* 5.1 and 10.7 Hz, 6′-H^a), 3.75 (1H, dd, *J* 5.1 and 10.7 Hz, 6′-H^b), 4.32 (2H, q, *J* 7.0 Hz, CH_2CH_3), 4.33 (2H, q, *J* 7.0 Hz, CH_2CH_3), 5.25 (1H, m, 1′-H), 5.90 (1H, ddd, *J* 1.8, 3.7 and 5.5 Hz, 3′-H), 6.13 (1H, ddd, *J* 2.2, 2.2 and 5.5 Hz, 2′-H), 7.69 (1H, s, 2-H), 8.32 (1H, br s, NH) and 11.06 (1H, br s, NH); δ_C ($CDCl_3$; 75.5 MHz) 14.1 ($NHCO_2CH_2CH_3$), 14.2 ($CO_2CH_2CH_3$), 35.0 (C-5′), 47.6 (C-4′), 60.5 ($NHCO_2CH_2CH_3$), 61.0 ($CO_2CH_2CH_3$), 63.4 (C-1′), 64.6 (C-6′), 125.4 (C-4), 130.0 (C-3′), 130.9 (C-2), 134.7 (C-2′), 138.4 (C-5), 152.7 ($NHCO_2CH_2CH_3$), 162.2 ($CO_2CH_2CH_3$) and 182.0 (NHCSNH); *m*/*z* (EI) 382.1341. $C_{16}H_{22}N_4O_5S$ requires *M*, 382.1311.

Ethyl (1′*R*,4′*S*)-5-(*N*′-ethoxycarbonyl-*S*-methylisothioureido)-1-[4′-(hydroxymethyl)cyclopent-2′-enyl]-1*H*-imidazole-4-carboxylate 22

Compound **22** (38.9 mg, 92%) was prepared from **21** (40.7 mg, 0.107 mmol) by the method described for **16**. Purification by silica gel column chromatography ($CHCl_3$–MeOH 10:1) gave *title compound* **22** as a colorless, waxy solid, ν_{max} (film)/cm^{-1} 3242, 1745, 1703, 1611 and 1225; $[\alpha]_D^{27}$ −77 (*c* 1.0 in $CHCl_3$); δ_H ($CDCl_3$) 1.26 (3H, t, *J* 7.3 Hz, CH_2CH_3), 1.37 (3H, t, *J* 7.3 Hz, CH_2CH_3), 1.61 (1H, ddd, *J* 5.5, 5.9 and 14.3 Hz, 5′-H^a), 2.48 (3H, s, SCH_3), 2.64 (1H, ddd, *J* 8.4, 8.8 and 14.3 Hz, 5′-H^b), 2.99 (1H, m, 4′-H), 3.63 (1H, dd, *J* 5.5 and 10.6 Hz, 6′-H^a), 3.69 (1H, dd, *J* 5.5 and 10.6 Hz, 6′-H^b), 4.18 (2H, q, *J* 7.3 Hz, CH_2CH_3), 4.34 (2H, q, *J* 7.3 Hz, CH_2CH_3), 5.17 (1H, m, 1′-H), 5.87 (1H, ddd, *J* 1.8, 2.2 and 5.5 Hz, 3′-H), 6.15 (1H, ddd, *J* 2.2, 2.2 and 5.5 Hz, 2′-H), 7.50 (1H, s, 2-H) and 8.04 (1H, br s, NH); δ_C ($CDCl_3$; 75.5 MHz) 14.1 (SCH_3), 14.3

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 cm^{-3}	CC_{50}[d]/μg cm^{-3}	EC_{50}	CC_{50}	EC_{50}	CC_{50}
1	7.0	440	> 500	> 500	27	> 100
2	240	> 500	> 500	> 500	> 100	> 100
6	11	300	155	> 500	13	56
7	> 500	> 500	> 500	> 500	> 100	> 100
8	4.8	> 500	> 500	> 500	> 100	> 100
9	4.1	> 500	> 500	> 500	21	> 100
23	42	> 500	320	> 500	41	> 100
24	170	> 500	175	> 500	36	> 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 cell proliferation using uptake of a tetrazolium dye (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 after 96 h for RT and MTT assays. [c] Effective concentration required to inhibit HIV-1 reverse transcriptase activity by 50%. [d] 50% Cytotoxic concentration.

($NHCO_2CH_2CH_3$), 14.7 ($CO_2CH_2CH_3$), 34.7 (C-5′), 47.7 (C-4′), 59.9 (C-1′), 60.4 ($NHCO_2CH_2CH_3$), 62.5 ($CO_2CH_2CH_3$), 64.7 (C-6′), 117.4 (C-4), 129.5 (C-3′), 133.2 (C-2), 138.7 (C-2′), 140.0 (C-5), 151.8 ($NHCO_2CH_2CH_3$), 158.6 [$NHC(SCH_3)$=N] and 163.4 ($CO_2CH_2CH_3$); *m*/*z* (EI) 396.1441. $C_{17}H_{24}N_4O_5S$ requires *M*, 396.1467.

(1′*R*,4′*S*)-5-Amino-3-[4′-(hydroxymethyl)cyclopent-2′-enyl]-imidazo[4,5-*d*][1,3]oxazin-7(3*H*)-one (carbocyclic 2′,3′-didehydro-2′,3′-dideoxyoxanosine) 23

A solution of compound **22** (25.9 mg, 0.0654 mmol) and 5 M methanolic KOH (1.0 cm^3) was refluxed at 90 °C for 30 min. The reaction solution was then adjusted to pH 3 with 4 M hydrochloric acid at 0 °C. The reaction mixture was concentrated to dryness and the residue was purified by silica gel column chromatography ($CHCl_3$–MeOH 5:1) to afford *title compound* **23** (9.1 mg, 56%) as a colorless, waxy solid, ν_{max} (film)/cm^{-1} 3330, 1771, 1651 and 1559; $[\alpha]_D^{20}$ −52 (*c* 0.5 in MeOH); δ_H (CD_3OD) 1.68 (1H, ddd, *J* 5.8, 5.8 and 13.9 Hz, 5′-H^a), 2.72 (1H, ddd, *J* 5.1, 8.8 and 13.9 Hz, 5′-H^b), 2.97 (1H, m, 4′-H), 3.57 (1H, dd, *J* 5.5 and 11.0 Hz, 6′-H^a), 3.63 (1H, dd, *J* 5.5 and 11.0 Hz, 6′-H^b), 5.45 (1H, m, 1′-H), 5.88 (1H, ddd, *J* 1.8, 2.2 and 5.5 Hz, 3′-H), 6.16 (1H, ddd, *J* 1.8, 2.2 and 5.5 Hz, 2′-H) and 7.74 (1H, s, 2-H); δ_C (CD_3OD; 75.5 MHz) 35.5 (C-5′), 49.1 (C-4′), 61.1 (C-1′), 65.4 (C-6′), 112.3 (C-7a), 130.5 (C-3′), 138.4 (C-2), 139.9 (C-2′), 154.2 (C-3a), 156.1 (C-5) and 161.6 (C-7); *m*/*z* (EI) 248.0906. $C_{11}H_{12}N_4O_3$ requires *M*, 248.0906.

(1′*R*,4′*S*)-5-Amino-3-[4′-(hydroxymethyl)cyclopentyl]imidazo-[4,5-*d*][1,3]oxazin-(3*H*)-one (carbocyclic 2′,3′-dideoxyoxanosine) 24

Compound **24** (9.1 mg, 92%) was prepared from **23** (9.8 mg, 0.0395 mmol), 10% Pd/C (1.0 mg), H_2 gas, and EtOH (2.0 cm^3) by the method described for compound **7**, in 14 h. Purification by silica gel column chromatography ($CHCl_3$–MeOH 5:1) provided *title compound* **24** as a colorless, waxy solid, ν_{max} (film)/cm^{-1} 3327, 1772, 1650 and 1558; λ_{max} (H_2O) 246 (ε 10 570) and 287 nm (ε 8100); λ_{max} (0.1 M HCl) 247 (ε 11 200) and 288 nm (ε 8230); λ_{max} (0.1 M NaOH) 274 nm (ε 8920); $[\alpha]_D^{22}$ −11 (*c* 0.5 in MeOH); δ_H (CD_3OD) 1.66–2.09 (4H, m, 3′- and 5′-H_2), 2.14–2.39 (3H, m, 2′-H_2 and 4′-H), 3.58 (2H, d, *J* 6.2 Hz, 6′-H_2), 4.64 (1H, m, 1′-H) and 7.83 (1H, s, 2-H); δ_C (CD_3OD; 75.5 MHz) 27.9 (C-3′), 32.3 (C-2′), 36.7 (C-5′), 41.6 (C-4′), 57.3 (C-1′), 66.6 (C-6′), 112.6 (C-7a), 138.7 (C-2), 154.5 (C-3a), 156.2 (C-5) and 161.4 (C-7); *m*/*z* (EI) 250.1049. $C_{11}H_{14}N_4O_3$ requires *M*, 250.1066.

Anti-HIV activity

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.

Oxanosine **1** showed anti-HIV activity with an EC_{50}-value of 7.0 μg cm^{-3} on human T cell leukemia CEM cells and 27 mg cm^{-3} in U937 cells, respectively. Compounds **8** and **9** were found to demonstrate stronger anti-HIV activity than **1** in CEM cells, with EC_{50}-values of 4.8 and 4.1 μg cm^{-3}, respectively, without cytotoxicity up to 500 μg cm^{-3}. These activities were comparable to that of ddI (EC_{50} 2.6 μg cm^{-3}). Carbocyclic oxanosine **2** showed no activity, but its derivatives **23** and **24** showed anti-HIV-1 activity with EC_{50}-values of 41 and 36 μg cm^{-3}, respectively, without cytotoxicity up to 100 μg cm^{-3} in U937 cells. Oxanosine derivatives were more potent than their carbocyclic counterparts.

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References

1 Preliminary account of part of this work: Y. Saito, M. Nakamura, T. Ohno, C. Chaicharoenpong, E. Ichikawa, S. Yamamura, K. Kato and K. Umezawa, *J. Antibiot.*, 2000, **53**, 309.
2 Isolation of oxanosine: (*a*) N. Shimada, N. Yagisawa, H. Naganawa, T. Takita, M. Hamada, T. Takeuchi and H. Umezawa, *J. Antibiot.*, 1981, **34**, 1216; (*b*) H. Nakamura, N. Yagisawa, N. Shimada, T. Takita, H. Umezawa and Y. Iitaka, *J. Antibiot.*, 1981, **34**, 1219.
3 Synthesis of oxanosine: (*a*) N. Yagisawa, T. Takita, H. Umezawa, K. Kato and N. Shimada, *Tetrahedron Lett.*, 1983, **24**, 931; (*b*) K.-C. Luk, D. W. Moore and D. D. Keith, *Tetrahedron Lett.*, 1994, **35**, 1007; (*c*) L. D. Napoli, G. D. Fabio, A. Messere, D. Montesarchio, G. Piccialli and M. Varra, *Tetrahedron Lett.*, 1998, **39**, 7397.
4 O. Itoh, S. Kuroiwa. S. Atsumi, K. Umezawa, T. Takeuchi and M. Hori, *Cancer Res.*, 1989, **49**, 996.
5 G. T. Crisp and B. L. Flynn, *Tetrahedron*, 1993, **49**, 5873.
6 K. Kato, N. Yagisawa, N. Shimada, M. Hamada, T. Takita, K. Maeda and H. Umezawa, *J. Antibiot.*, 1984, **37**, 941.

7 M. Yoshikawa, T. Kato and T. Takenishi, *Tetrahedron Lett.*, 1967, **7**, 5065.
8 H. Kurata, S. Nishiyama, S. Yamamura, K. Kato, S. Fujiwara and K. Umezawa, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 283.
9 E. J. Hutchinson, B. F. Taylor and G. M. Blackburn, *Chem. Commun.*, 1996, 2765.
10 D. H. Robinson and G. Shaw, *J. Chem. Soc.*, *Perkin Trans. 1*, 1972, 1715.
11 Synthesis of oxazinone ring by similar procedures: (*a*) H. Matsumoto, C. Kaneko, T. Mori and Y. Mizuno, *Chem. Pharm. Bull.*, 1989, **37**, 229; (*b*) B. K. Bhattacharya, R. K. Robins and G. R. Revankar, *J. Heterocycl. Chem.*, 1990, **27**, 787. See also ref. 3*a*.