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FIRST SYNTHESIS OF ENANTIOMERICALLY PURE CARBOCYCLIC OXANOSINE AS A POTENTIAL CHEMOTHERAPEUTIC AGENT

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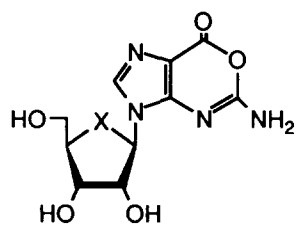
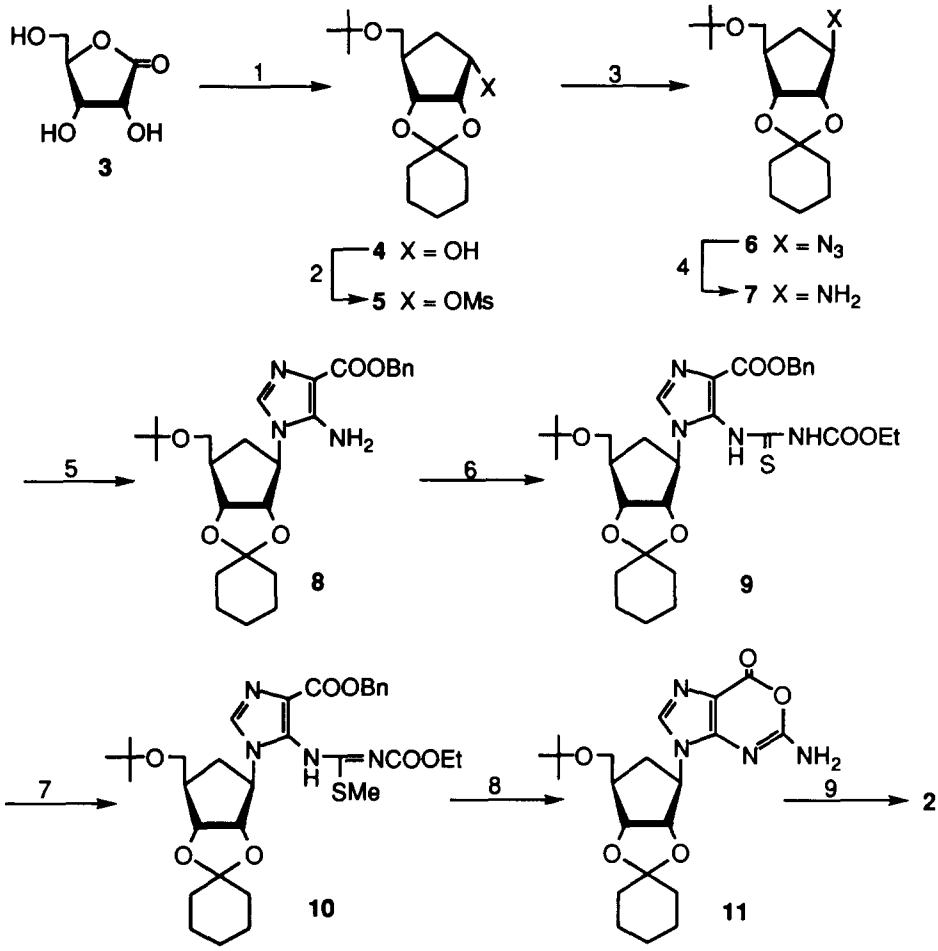
Abstract: The first synthesis of optically active carbocyclic oxanosine **2** has been achieved in 14 steps from commercially available D-ribonic acid γ-lactone. When evaluated for the inhibition activity of NGF-induced differentiation on PC12 cells, **2** was about 10-fold less active than natural oxanosine.

Oxanosine **1**, a novel nucleoside antibiotic isolated from the culture broth of *Streptomyces capreolus* MG265-CF3, inhibits the growth of HeLa cells in culture and suppresses the growth of L1210 leukemia in mice.[1] Furthermore, **1** has proved to lower the intracellular level of guanine nucleotides and specifically inhibit differentiation mediated by G-proteins including Ras.[2] Recently, **1** was found to alter tumor cell morphology into the normal morphology in temperature sensitive Kirsten sarcoma virus-infected rat kidney (K-ras^{ts}-NRK) cells [3] and inhibit nerve growth factor (NGF)-induced morphological and enzymatic differentiation in rat pheochromocytoma PC12h cells.[4] These noteworthy biological activities of **1** prompted our interest in the synthesis of carbocyclic analog of **1** with the hope that it might be metabolically more stable [5] and selective in its biological activity, as carbocyclic nucleosides have emerged as a promising group of compounds for drug discovery in the anti-tumor and anti-viral fields. In this report we describe the first synthesis and the inhibition activity of NGF-induced differentiation on PC12 cells of the enantiomerically pure carbocyclic oxanosine **2**.



1 X = O Oxanosine
2 X = CH_2 Carbocyclic oxanosine

As shown in Scheme 1, the synthesis of the optically active carbocyclic oxanosine **2** began with the chiral alcohol **4** prepared from D-ribonic acid γ-lactone **3** according to the protocol of Borchardt *et al.* [6] and the formation of oxazinone ring was accomplished by the methodology developed by one of the authors on the occasion of the total synthesis of **1**.[7]



Scheme 1 *Reagents and Conditions*: 1) 6 steps. See ref.5; 2) MsCl, Et_3N, CH_2Cl_2, 0 °C,1 h; 3) NaN_3, DMF, 120 °C, 18 h; 4) $LiAlH_4$, THF, 0 °C, 2.5 h; 5) EtO-CH=N-CH(CN)COOBn, EtOH, reflux, 30 min; 6) EtOCONCS, CH_3CN, reflux, 2 h; 7) 0.1 N-NaOH, MeI, rt, 2 h; 8) 5N-KOH, MeOH, reflux, 30 min; 9) CF_3COOH-H_2O (2:1), 50 °C, 3 h.

The alcohol **4** was converted to the mesylate **5** with methanesulfonyl chloride in 95 % yield. Displacement with NaN_3 in DMF gave the azide **6** in 84 % yield. Reduction of **6** with $LiAlH_4$ afforded the amine **7** in 70 % yield. Reaction of compound **7** with ethyl *N*-(benzyloxycarbonylcyanomethyl)formimidate furnished the imidazole **8** in 55 % yield. Then, compound **8** was reacted with ethoxycarbonyl isothiocyanate to give the

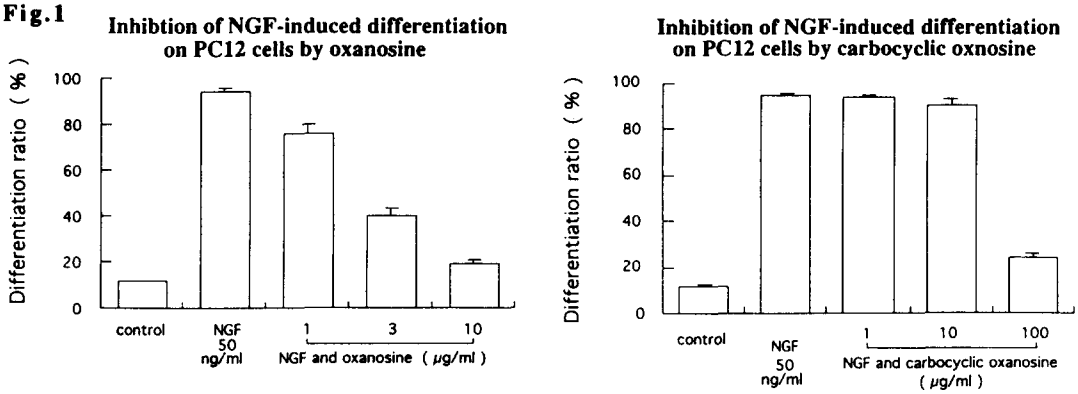
thiourea **9** in 91 % yield, which with methyl iodide in dilute sodium hydroxide yielded the methylthio derivative **10** in 92 % yield. Cyclization of compound **10** with 5N-methanolic KOH under reflux for 30 min followed by neutralization of the reaction mixture with 2N-HCl provided the oxazinone **11**[8] in 73 % yield. Finally, deprotection of **11** by heating in CF_3COOH/H_2O (2:1) at 50 °C afforded the target compound **2**[9] in 81 % yield.

Inhibition studies of NGF-induced morphological differentiation:

The signal transduction through NGF receptor to induce differentiation in PC12 cells is known to include c-Ras function, since microinjection of anti-Ras inhibits NGF-induced differentiation in PC12 cells.[10] Recently, we also found that oxanosine **1** inhibited NGF-induced but not dibutyl cyclic AMP-induced differentiation of PC12h cells.[4] In this assay, carbocyclic oxanosine **2** did not induce flat morphology markedly in K-ras^{ts}-NRK cells, but as shown in Fig.1, **2** inhibited the NGF-induced morphological differentiation at about 10 times higher concentration than that of **1**. Thus **2** inhibited the c-Ras activity but not the activated Ras activity in cultured cells.

Inhibition of NGF-induced neurite formation by carbocyclic oxanosine in PC12h cells

Fig.1



Legend for Fig.1:

PC12h cells were incubated with oxanosine or carbocyclic oxanosine for 48 hrs in medium containing 0.2% semifetal calf serum. Cells with neurites were scored under the phase contrast microscope.

In summary, we have developed the first synthesis of carbocyclic oxanosine **2** from the readily available chiral cyclopentylalcohol **4** and have found that **2** inhibited the NGF-induced morphological differentiation at about 10 times higher concentration than that of oxanosine **1**. **2** should be further pursued for its therapeutic potential as an anti-tumor and/or an anti-viral agent.

Acknowledgment:

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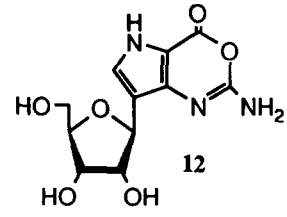
5. It has been found that the oxazinone ring in **1** is gradually hydrolyzed in mammalian sera to yield the bioinactive products. In an effort to prevent this enzymatic hydrolysis, 3-deazaoxanosine **12** has been prepared. Although **12** was resistant to the hydrolytic enzyme of mouse serum, it was much less active than **1** as an anti-tumor agent. Niitsuma, S.; Kato, K.; Takita, T.; Umezawa, H. *Tetrahedron Lett.* **1985**, *26*, 5785.



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7. Yagisawa, N.; Takita, T.; Umezawa, H.; Kato, K.; Shimada, N. *Tetrahedron Lett.* **1983**, *24*, 931. See also Luk, K-C; Moore, D. W.; Keith, D. D. *Tetrahedron Lett.* **1994**, *35*, 1007.

8. Selected spectroscopic data for **11**: colorless foam, $[\alpha]^{26}_D$ -34° (*c* 0.57, $CHCl_3$); λ_{max} (CH_3CN) 246 nm (ε 8,900), and 283 nm (ε 4,700); ^1H NMR (270 MHz, $CDCl_3$) δ 1.20 (9H, s), 1.20-1.80 (10H, complex), 2.13 (1H, dt, J=10.8 and 11.1 Hz, 5'-H), 2.43 (2H, complex, 4'-H, 5'-H), 3.48 (2H, complex, 6'-H), 4.57 (2H, complex, 1'-H, 3'-H), 4.80 (1H, t, J=6.3 Hz, 2'-H), 5.81 (2H, br s, NH2), and 7.65 (1H, s); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ 23.5, 24.0, 25.0, 27.5, 34.2, 34.6, 37.5, 43.9, 61.4, 62.1, 73.0, 81.1, 83.9, 113.0, 114.0, 137.1, 152.3, 154.3, and 158.7; HRMS m/z 418.2214 calcd for $C_{21}H_{30}N_4O_5$, found 418.2245.

9. Selected spectroscopic data for **2**: colorless foam, $[\alpha]^{21}_D$ -20° (*c* 1.16, H_2O); λ_{max} (H_2O) 248 nm (ε 4,800), and 288 nm (ε 3,900); ^1H NMR (270 MHz, D_2O) δ 1.89 (1H, ddd, J=8.7, 10.4, and 13.0 Hz, 5'-H), 2.38 (1H, m, 4'-H), 2.57 (1H, dt, J=8.4 and 13.0 Hz, 5'-H), 3.84 (2H, d, J=6.3 Hz, 6'-H), 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); ^{13}C NMR (100.5 MHz, D_2O) δ 29.4, 45.6, 60.1, 63.9, 72.7, 76.1, 112.3, 139.7, 154.4, 157.5, and 160.5; MS m/z 283 (M^++1); HRMS m/z 266.0776 calcd for $C_{11}H_{12}N_3O_5$ (M^+-NH_2), found 266.0733.

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