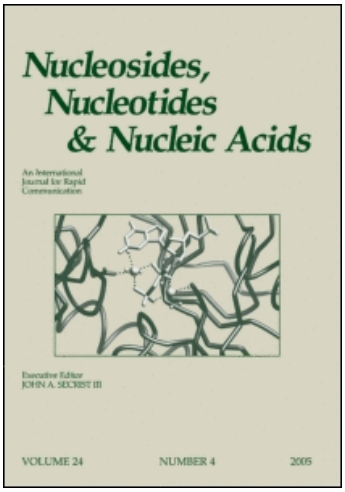

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A 5'-Noraristeromycin Enantiomer with Activity Towards Hepatitis B Virus

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A 5'-Noraristeromycin Enantiomer with Activity Towards Hepatitis B Virus

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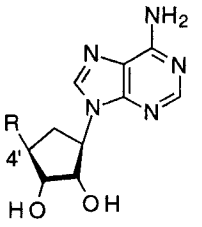
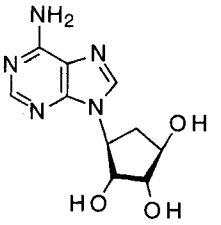
Abstract

(+)-5'-Noraristeromycin has selective activity against hepatitis B virus (HBV) replication in 2.2.15 cells in culture, while the (-) enantiomer was found to be inactive. A modified synthesis is presented for (+)-5'-noraristeromycin.

Beginning with the synthesis of carbocyclic adenosine (aristeromycin, **1**) in 1966,[1] a number of carbocyclic nucleosides have been studied as potential medicinal and biochemical agents.[2] Several years ago, we reported[3] the first carbocyclic nucleoside lacking the C-5' carbon and this commenced concerted investigations by us[4] and others[5] into this class of compounds now known as 5'-nor carbocyclic nucleosides. The adenosine derivative (5'-noraristeromycin) has been shown to possess notable antiviral activity (**2**)[4b] and anti-trypanosomal activity (**3**)[6] with no undesirable toxic side effects. In exploring the antiviral range of 5'-noraristeromycin, enantiomers **2** and **3** were tested for their potential towards hepatitis B virus.



1, R=CH_2OH
2, R=OH



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Chemistry

The synthesis of both **2**[7] and **3**[8] have been reported and, while the literature method[7] was used for **2**, a more direct route to **3** than previously described[8] was employed. In that regard, the synthesis of **3** was accomplished by, first, the palladium-catalyzed coupling of **4**[8] with N^6-benzoyladenine[9] to afford **5**. Subsequent debenzoylation of **5** using ammonium hydroxide in methanol to **6** was followed by glycolization with osmium tetroxide to give **3**.

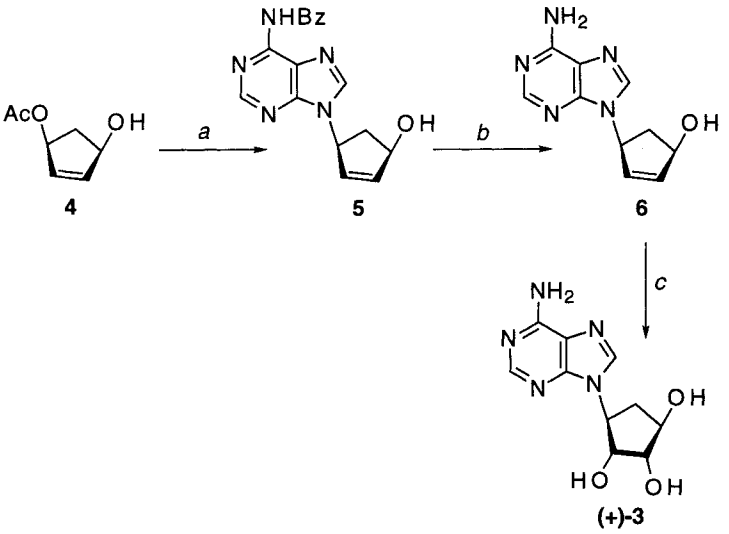
Antiviral Results

Antiviral analyses for the inhibition of HBV replication were performed on confluent cultures of the chronically HBV-producing human hepatoblastoma cell line, 2.2.15, as previously described.[10] Cells were treated with 9 consecutive daily doses (medium removed daily) of the agents. Antiviral activity was assessed by dot blot hybridization analysis for reductions in the levels of extracellular HBV virion DNA and cytotoxicity by uptake of neutral red dye.[10] In these analyses, reductions in virion production of less than 3-fold are routinely not statistically significant.[10]

(+)-5'-Noraristeromycin (compound **3**) was found to inhibit intracellular HBV replication and virion production in 2.2.15 cells at a concentration significantly below the 50% cytotoxic concentration [CC_{50}] (see Table below). The (-) enantiomer of **3** and the dideoxy intermediate (compound **6**) were inactive against HBV replication.

Experimental. Melting points were recorded on a Meltemp II melting point apparatus and are uncorrected. Combustion analyses were performed by M-H-W Laboratories, Phoenix AZ. ^1H and ^{13}C spectra were recorded on a Bruker AC 250 spectrometer (operated at 250 and 62.5 MHz, respectively) all referenced to internal tetramethylsilane (TMS) at 0.0 ppm. The spin multiplicities are indicated by the symbols s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br (broad). The optical rotation was measured on a JASCO DIP-370 polarimeter. Reactions were monitored by thin-layer chromatography (TLC) using 0.25 mm Whatman Diamond silica gel 60-F_{254} precoated plates with visualization by irradiation with a Mineralight UVGL-25 lamp or exposure to iodine vapor. Column chromatography was performed on Whatman silica, 230-400 mesh, 60 Å and elution with the indicated solvent system. Yields refer to chromatographically and spectroscopically (^1H and ^{13}C NMR) homogeneous materials.

Abbreviations: MeOH, methanol; PPh_3, triphenylphosphine; DMSO, dimethylsulfoxide; DMSO-d_6, deuterated dimethyl sulfoxide; NH_4OH, ammonium hydroxide; THF, tetrahydrofuran; EtOAc, ethyl acetate; CH_2Cl_2, methylene chloride; NaH, sodium hydride, DMF, dimethylformamide; NMO, 4-methylmorpholine *N*-oxide.



Reaction conditions: *a*, N^6-benzoyladenine and NaH in DMSO then add **4**/$(Ph_3P)_4Pd/PPh_3$ in THF at rt then 55°C; *b*, NH_4OH/ MeOH, 120°C, 2 days; *c*, OsO_4/60% aq. 4-methylmorpholine N-oxide in THF/H_2O.

Scheme 1

Table 1

		Extracellular HBV virion DNA		Intracellular HBV replication intermediates	
Compound	CC_{50} (μM)	EC_{50} (μM)	EC_{90} (μM)	EC_{50} (μM)	EC_{90} (μM)
(+)-3	446 ± 20	1.4 ± 0.1	9.6 ± 0.8	15 ± 1.1	41 ± 3.0
(-)-3	422 ± 34	>10	>10	-	-
6	132 ± 12	>10	>10	-	-

(1*S*,4*R*)-*N*-[9-(4-Hydroxy-2-cyclopentenyl)-9*H*-purin-6-yl]benzamide (5). To a solution of N^6-benzoyladenine (3.37 g, 14.10 mmol) in anhydrous DMSO (30 mL) was added NaH (95%, 0.39 g, 15.4 mmol). The mixture was stirred for 30 min under an argon atmosphere. To this mixture was added tetrakis(triphenylphosphine)palladium (1.01 g, 0.87 mmol), Ph_3P (0.39 g, 1.49 mmol) and a solution of **4**[8] (2.0 g, 14.08 mmol) in anhydrous THF (30 mL). The mixture was stirred at 55 °C for 2

days. The volatiles were removed by rotary evaporation. The residue was slurried in CH_2Cl_2 and filtered. The filtrate was evaporated, and the residue was purified via column chromatography eluting with EtOAc, followed by EtOAc/MeOH (12:1). Product-containing fractions were combined and evaporated to afford an off-white foam, which was recrystallized with hexane-EtOAc to give **5** (2.65 g, 59 %) as a white solid: mp 159-160°C (lit.[8] 159-161°C). The spectral data is consistent with that reported[8] previously for **5**. The microanalytical data confirmed the purity of the product.

(1*S*,4*R*)-4-Hydroxy-1-(6-amino-9*H*-purin-9-yl)cyclopent-2-ene (6). A solution of **5** (2.5 g, 7.78 mmol) in 200 mL concentrated ammonia/MeOH (1:1) was sealed in a steel bomb and heated at 100 °C for 2 days. The bomb was cooled. The solvent was removed and the tan residue was triturated in MeOH, filtered and purified via column chromatography eluting with CH_2Cl_2/MeOH (98:2 then 95:5) to afford 1.35 g (80 %) of **6** as an off white solid, mp 189-190 °C; ^{1}H NMR (DMSO-d_6) δ 1.73-1.82 (dt, 1H), 2.86-2.95 (m, 1H), 4.75 (br, 1H), 5.51 (m, 1H), 5.60 (d, 1H), 6.20 (d, 1H), 6.22 (d, 1H), 7.32, (br, 2H), 8.11 (s, 1H), 8.19 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 41.14, 57.16, 73.78, 119.03, 130.73, 135.55, 139.32, 148.86, 152.22, 156.06. Calcd. for $C_{10}H_{11}N_5O$: C, 55.47; H, 5.08; N, 32.11. Found: C, 55.43, H, 5.30, N, 32.20.

(1*R*, 2*S*, 3*R*, 4*S*)-4-(6-Amino-9*H*-purin-9-yl)cyclopentane-1,2,3-triol ((+)-3). A solution of **6** (1.35 g, 6.19 mmol) in 50 mL THF/H_2O (10:1) was treated with OsO_4 (100 mg) and NMO (1 mL) and the solution stirred at rt overnight. The solvents were evaporated under reduced pressure and the residue triturated with MeOH, filtered and the tan solid purified further by column chromatography eluting with CH_2Cl_2/MeOH (5:1, then 1:1) to afford 0.86 g (55 %) of **3** as a white solid: mp 251-252 °C; $[\alpha]^{23}_D$ +45.869° (*c* 0.14, DMF); ^{1}H NMR (DMSO-d_6) δ 1.77-1.90 (dq, 1H), 2.57-2.66 (m, 1H), 3.76 (br, 1H), 3.90 (br, 1H), 4.52 (br, 1H), 4.63-4.73 (q, 1H), 4.90 (br s, 1H), 5.02 (br s, 1H), 5.37 (br s, 1H), 7.23 (br s, 2H), 8.12 (s, 1H), 8.16, (s, 1H); ^{13}C NMR (DMSO-d_6) δ 36.49, 58.47, 73.71, 75.25, 76.73, 119.24, 140.00, 149.40, 151.99, 156.04. Calcd. for $C_{10}H_{13}N_5O_3$: C, 47.80; H, 5.21; N, 27.88. Found: C, 47.92, H, 5.41, N, 27.88.

Acknowledgments

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