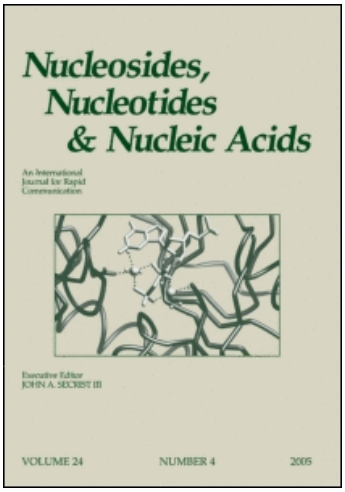

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An Efficient, Short Synthesis, and Potent Anti-Hepatitis B Viral Activity of a Novel Ring-Expanded Purine Nucleoside Analogue Containing a 5:7-Fused, Planar, Aromatic, Imidazo[4,5-*E*][1,3]diazepine Ring System

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AN EFFICIENT, SHORT SYNTHESIS AND POTENT ANTI-HEPATITIS B VIRAL ACTIVITY OF A NOVEL RING-EXPANDED PURINE NUCLEOSIDE ANALOGUE CONTAINING A 5:7-FUSED, PLANAR, AROMATIC, IMIDAZO[4,5-*E*][1,3]DIAZEPINE RING SYSTEM

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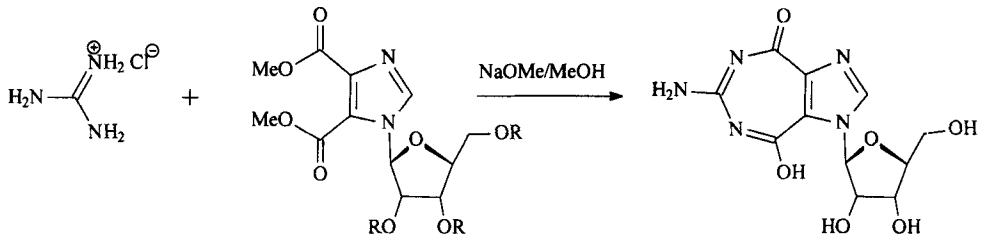
Abstract: An efficient, short synthesis of a ring-expanded nucleoside analogue containing a novel 5:7-fused, planar, and potentially aromatic imidazo[4,5-*e*][1,3]diazepine heterocyclic ring system is reported. The target compound, 6-amino-8-hydroxy-4*H*-1-β-D-ribofuranosylimidazo[4,5-*e*][1,3]diazepin-4-one (**2**) was synthesized in a single step in ≥90% yield by condensation of guanidine with either methyl 1-β-D-ribofuranosylimidazole-4,5-dicarboxylate (**1a**) or its 2',3',5'-tri-*O*-benzoyl derivative (**1b**). Compound **2** showed potent anti-hepatitis B virus (anti-HBV) activity with an EC_{50} value of 0.17 μM in the transfected hepatoma cell line 2.2.15, and a low cellular toxicity with a CC_{50} value of 2.4 mM (TI >14,000).

We have been involved in a broad research program aimed at exploring the chemistry, biochemistry, and biomedical applications of novel ring-expanded purine nucleosides.[1] As part of this program, we now report an efficient, short synthesis of the title 5:7-fused, planar, and potentially aromatic, ring-expanded purine nucleoside analogue containing the imidazo[4,5-*e*][1,3]diazepine heterocyclic ring system. We also report here the observed potent *in vitro* anti-hepatitis B virus activity of the target compound.



Guanidine.HCl **1** **2**

a; R = H
b; R = Bz

Scheme 1

The target compound, 6-amino-8-hydroxy-4*H*-1-β-D-ribofuranosylimidazo[4,5-*e*][1,3] diazepin-4-one (**2**) was synthesized in a single step in ≥90% yield by condensation of guanidine with either methyl 1-β-D-ribofuranosylimidazole-4,5-dicarboxylate (**1a**)[2] or its 2',3',5'-tri-*O*-benzoyl derivative (**1b**)[2] (**Scheme I**). Nucleoside **2** was characterized by ^{1}H NMR, elemental microanalyses, and mass spectral data. While several tautomeric structures are possible for **2**, only one of them has been shown in Scheme I.

The target nucleoside **2** was screened[3] for anti-hepatitis B virus (anti-HBV) activity in the transfected hepatoma cell line 2.2.15. Nucleoside **2** showed potent anti-HBV activity in this cell line, with an EC_{50} value of 0.17 μM and a low cellular toxicity, with a CC_{50} value of 2.4 mM (therapeutic index, TI >14,000). The observed activity and toxicity data for 3TC (β-L-oxathiolane-cytosine)[4], used as a reference standard for anti-HBV activity, are EC_{50} = 0.065 μM and CC_{50} = 2.0 mM, respectively.

EXPERIMENTAL SECTION

^{1}H NMR spectra were recorded on a General Electric QE-300 (300 MHz) instrument. The spectral data are reported in the following format: chemical shift (all relative to Me_4Si as an internal reference standard unless otherwise indicated), multiplicity (s = singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, m = multiplet, b = broad), integration, coupling constants, exchangeability after D_2O addition, and assignment of resonances. Elemental Microanalyses were performed by Atlantic Microlab, Inc., Norcross,

Georgia. The mass spectra were recorded at the Mass Spectral Facility, Department of Biochemistry, Michigan State University. Thin layer chromatography was performed on Merck Kieselgel 60 GF_{254} plates (0.2 mm thickness). Melting points were determined on a Thomas-Hoover capillary melting point apparatus, and are uncorrected.

6-Amino-8-hydroxy-4H-1-β-D-ribofuranosylimidazo[4,5-e][1,3]diazepin-4-one (2). Guanidine hydrochloride (0.38 g, 4 mmol) was added to 4 mL of 2.3 M NaOMe solution resulting from sodium (0.75 g) dissolved in 15 mL of absolute methanol. The mixture was stirred in an ice bath for 30 min. The precipitated sodium chloride was removed by filtration, and the filtrate was poured into a solution of either methyl 1-β-D-ribofuranosylimidazole-4,5-dicarboxylate (**1a**)[2] or methyl 1-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)imidazole-4,5-dicarboxylate (**1b**)[2] (1 mmol) in 20 mL of absolute methanol. The mixture was stirred at room temperature for 24-48 h when a TLC analysis showed that the reaction was complete. The reaction mixture was filtered if necessary, and the clear filtrate was neutralized with 2M hydrochloric acid. The resulting precipitate was filtered and washed with water and methanol to give product **2** (0.28 g, 90%) as a white solid. An analytically pure sample of **2** was obtained by preparative TLC of the above-mentioned clear filtrate, before neutralization with hydrochloric acid, on a preparative silica gel plate, using chloroform - methanol - 30% ammonium hydroxide (2:2:1) as the developing solvent: Rf 0.35, mp >250 °C; ^{1}H NMR (DMSO-d_6) δ 10.64 (brs, 1H, OH, exchangeable with D_2O), 8.59 (s, 1H, imidazole), 7.47 (brs, 1H, NH, exchangeable with D_2O), 6.60 (brs, 1H, NH, exchangeable with D_2O), 6.40 (d, 1H, *J*=2.4 Hz, 1'-H), 5.44 (d, 1H, *J*=3.9 Hz, OH, exchangeable with D_2O), 5.17 (t, 1H, *J*=4.5 Hz, OH, exchangeable with D_2O), 5.06 (d, 1H, *J*=4.5 Hz, OH, exchangeable with D_2O), 4.07 (m, 2H, 2' and 3'-H), 3.91 (m, 1H, 4'-H), 3.73 (dd, 1H, *J*=12.3 and 2.7 Hz, 5'-H_1), 3.59 (dd, 1H, *J*=12.3 and 3.0 Hz, 5'-H_2); FABMS *m/z*: 312 [MH^+].

Anal. Calcd. for $C_{11}H_{13}N_5O_6 \cdot 1.25H_2O$: C, 39.58; H, 4.68; N, 20.98. Found: C, 39.58; H, 4.65; N, 20.85.

Antiviral Screening for Hepatitis B Virus Activity. The anti-HBV assays of **2** were performed[3a] according to the published protocol of Korba and Milman,[3b] using cultures of 2.2.15 cells. In brief, the assay consisted of (a) seeding the chronically HBV-producing

human liver cells[5] into 24-well tissue culture plates, and growing to confluence, (b) adding test compounds daily for a continuous 9-day period., and then collecting the culture medium (changed daily during the treatment period), and storing for analysis of extracellular (virion) HBV DNA after 0,3,6, and 9 days of treatment, (c) lysing treated cells 24 hours following day 9 of treatment for the analysis of intracellular HBV genomic forms, and (d) analyzing HBV DNA both *qualitatively* and *quantitatively* for overall levels of HBV DNA (both extracellular and intracellular DNA) and the relative rate of HBV replication (intracellular DNA).

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