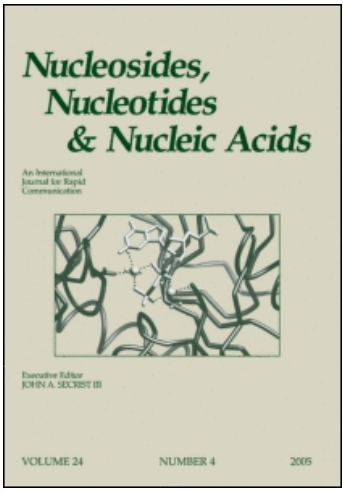

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Potent Anti-hepatitis B Viral Activity and Inhibition of Bacteriophage T7 RNA Polymerase by a "Fat" Nucleoside and Its 5'-Triphosphate Derivative: Synthetic, Biochemical, and Biological Studies of 4,8-Diamino-6-imino-6*h*-1-β-D-ribofuranosylimidazo[4,5-*E*][1,3]diazepine-5'-triphosphate

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POTENT ANTI-HEPATITIS B VIRAL ACTIVITY AND INHIBITION OF BACTERIOPHAGE T7 RNA POLYMERASE BY A "FAT" NUCLEOSIDE AND ITS 5'-TRIPHOSPHATE DERIVATIVE: SYNTHETIC, BIOCHEMICAL, AND BIOLOGICAL STUDIES OF 4,8-DIAMINO-6-IMINO-6*H*-1-β-D-RIBOFURANOSYL-IMIDAZO[4,5-*E*][1,3]DIAZEPINE-5'-TRIPHOSPHATE

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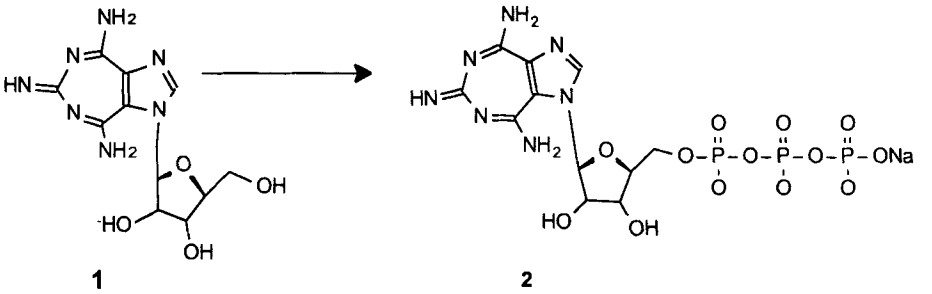
***Abstract**: The title nucleoside, 4,8-diamino-6-imino-6H-1-β-d-ribofuranosylimidazo[4,5-e][1,3]-diazepine, exhibited potent anti-hepatitis B viral activity with minimum toxicity in vitro, and its 5'-triphosphate derivative strongly inhibited the bacteriophage T7 RNA polymerase.*

Ring-expanded ("fat") nucleosides and nucleotides are potentially useful probes for nucleic acid metabolism, structure, and function. With their structural resemblance to natural purines, they are a rich source of substrates or inhibitors of enzymes of purine metabolism as well as of those requiring energy cofactors such as ATP or GTP. As ring-expansion is anticipated to significantly affect the base-ribose spatial geometry, sugar pucker, and syn/anti conformational array, they are excellent tools for investigations of steric and conformational constraints of nucleic acid double-helices. Furthermore, from a medicinal standpoint, ring-expanded nucleosides have potential utility in viral and cancer chemotherapy.

We have recently discovered that a number of ring-expanded nucleosides that we have reported earlier possess potent anti-hepatitis B viral activity in tissue culture systems.[1] Included in this list is the diamino-imino-substituted nucleoside **1**, whose synthesis we reported in 1994.[2] Compound **1** exhibited anti-HBV activity in the transfected hepatoma cell line 2.2.15, with an EC_{50} value of 0.397 μM and a CC_{50} value of 500 μM (SI>1250). Nucleoside **1** also inhibited the synthesis of HBV core antigen (HbcAg) in the above cell line, an activity apparently unique to this class of compounds. This nucleoside, although not the most potent of all the "fat" nucleosides that exhibited anti-hepatitis B viral activity, was nevertheless the compound of choice as a prototype for further explorations into the mechanism of action of antiviral activity of ring-expanded nucleosides containing analogous heterocyclic ring systems. The choice of **1** for further biochemical studies was based upon several factors, including its ease of synthesis, relatively high stability under normal biochemical and physiological conditions, planarity and potential aromaticity to render favorable



1 2

hydrophobic interactions compatible with those of natural nucleosides, and its capability to exist in several tautomeric forms in solution, mimicking adenine, guanine, isoguanine, and 2,6-diaminopurine. We report herein the synthesis and some biochemical studies of the 5'-triphosphate **2**, derived from **1**.

The 5'-triphosphate **2** was prepared from **1** using standard reagents and procedures.[3] One of the biochemical experiments was a comparative study of the synthesis of an RNA transcript by bacteriophage T7 RNA polymerase[4] from a 37-nucleotide-long DNA template (**I**) that is annealed to a 17-nucleotide-long promoter of the said polymerase, in the presence or absence of **2**. After separation of the full-length (20 nucleotide) transcripts and the shorter 'abortive' fragments by polyacrylamide-urea gel electrophoresis, the products were visualized by autoradiography.

(a) The 37-mer (**I**):

5' T AAT ACG ACT CAC TAT A.....(T7 RNA Promoter)

3' A TTA TGC TGA GTG ATA TCC TGA TCG CCT CCG ATC AGG 5'

(b) the 42-mer (**II**)

5' T AAT ACG ACT CAC TAT A.....(T7 RNA Promoter)

3' A TTA TGC TGA GTG ATA TCG GAA GGA AGC ACG TGG GAG CTT AA 3'

The observed significant reduction in the intensity of bands of the full-length polymer in the presence of **2**, as determined by (laser) scanning densitometry of autoradiograms of the [α-^{32}P]GTP-labeled transcripts that were analyzed by gel electrophoresis or with the use of a phosphoimager screen, suggests that **2** is a potent inhibitor of T7 RNA polymerase. The experiment was repeated using a different template (**II**) that was 42-nucleotide-long, along with the same 17-nucleotide promoter used before. The results once again corroborated the finding that **2** is a potent inhibitor of T7 RNA polymerase.

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