The Synthesis and Biophysical Investigations of Novel Ring-Expanded Nucleosides, Nucleotides, and Homopolymers Containing the 5:7-Fused Heterocyclic Ring System Imidazo[4,5-*e*][1,4]diazepine

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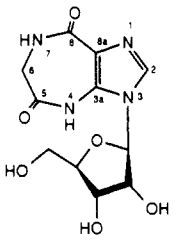
The synthesis and conformational studies of two isomeric 5:7-fused nucleosides, 4,5,7,8-tetrahydro-6*H*-3-(β-D-ribofuranosyl)imidazo[4,5-*e*][1,4]diazepine-5,8-dione (**1**) and its 1-glycosyl analogue (**2**), their respective 5′-mono- and -diphosphate derivatives (**13** and **14**), and the corresponding homopolymers (**15** and **16**) are presented. While **1** is devoid of activity against murine leukemia virus (MuLV) in tissue culture systems, **2** is as active as interferon. Nucleoside **1** exhibits a predominantly syn base–ribose conformation both in solid state and solution. The conformation of **2**, by contrast, is anti in both phases. The sugar pucker geometry in **1** is $C_{2'\text{-endo}}$–$C_{3'\text{-exo}}$, whereas that in **2** is the opposite $C_{2'\text{-exo}}$–$C_{3'\text{-endo}}$. The 5′-diphosphate derivative of the anti conformer (**14c**) undergoes polymerization with *E. coli* polynucleotide phosphorylase with ease, and the resultant homopolymer **16** reveals considerable internal secondary structure with a stable helical conformation. The corresponding observations on the syn conformer are strikingly opposite.

Introduction

It has recently been reported[1] that a correlation might exist between the preferred sugar conformation and the activity of nucleoside analogues against the human immunodeficiency virus. Systematic studies are warranted for exploring the little understood interrelationships of nucleoside base–sugar conformation, sugar pucker, ease of in vivo phosphorylation, enzymic polymerization, and biological activity. Likewise, it is important to investigate the consequences of aberrant nucleoside conformation (primary structure) on the ease of formation of helix, helical structure, stability, and conformation of polynucleotides (secondary structure). Both primary and secondary structures may play important roles in the determination of the final outcome of biological activity of nucleoside analogues. In this regard, ring-expanded nucleoside analogues such as **1** and **2** are excellent probes for exploring the above interrelationships by virtue of their unique structural features and steric constraints, coupled with their potentially novel electronic, ionic, and conformational characteristics. Furthermore, with their structural resemblance to the natural counterparts, ring-expanded nucleo(s/t)ides are a potentially rich source of substrates or inhibitors of enzymes of the purine biosynthetic pathway as well as of those requiring ATP or GTP energy cofactors. Apart from their anticipated biochemical and biophysical significance, they are also of interest from a strictly chemical standpoint,[2] e.g. their relative thermodynamic stability, (anti/non)aromaticity, acid–base property, and opportunistic rearrangements.[3b-d]

We report here the synthesis and conformational studies of two regioisomeric ring-expanded xanthosine analogues **1** and **2**, their corresponding 5′-mono- and -diphosphate derivatives, and their respective nucleotide homopolymers, all containing the 5:7-fused heterocyclic base system, imidazo[4,5-*e*][1,4]diazepine.[3a,f,4a,b] In addition, the results of preliminary biological screening of **1** and **2** against a retrovirus are presented. The determination of three dimensional structures of **1** and **2** by X-ray crystallography aided not only in the intended conformational studies but also in verification of the regioisomeric and anomeric assignments. Besides, in light of a number of alleged seven-membered ring heterocycles whose structures were later found to be in error,[4c-e] structural confirmation of **1** and **2** by single-crystal X-ray diffraction was especially warranted.



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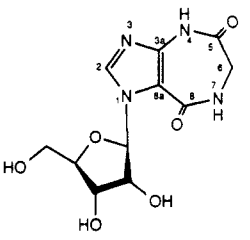

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To date, the only widely studied 5:7-fused nucleosides are the naturally occurring synergistic antitumor antibiotics coformycin (**3a**)[5a-c] and pentostatin (2′-deoxyco-

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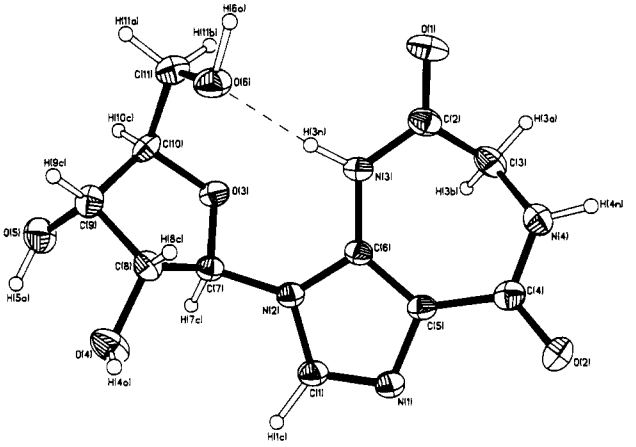


Figure 1. ORTEP view of **1** showing the atom numbering scheme and thermal ellipsoids at the 30% probability level.

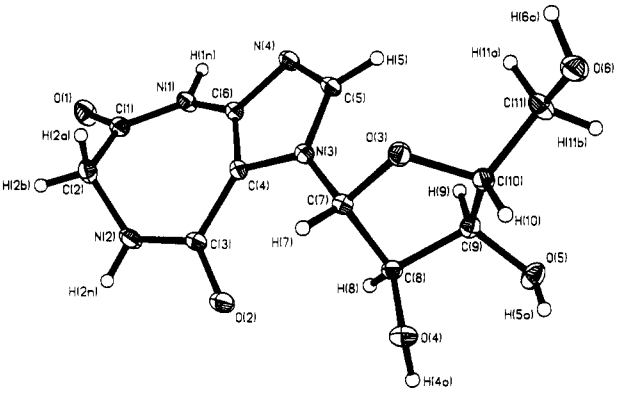
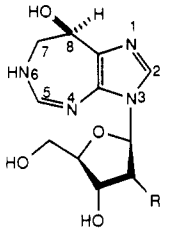


Figure 2. ORTEP view of **2** showing the atom numbering scheme and thermal ellipsoids at the 30% probability level.

formycin, **3b**)[5d-h] which contain the imidazo[4,5-*d*][1,3]-diazepine ring system, their synthetic analogues containing either a modified sugar[5c] or a modified imidazole ring,[6] and azepinomycin (**4**)—a non-nucleoside—which contains the title imidazo[4,5-*e*][1,4]diazepine ring skeleton.[7] Other



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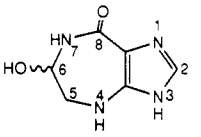
R = OH, Coformycin
R = H, Pentostatin



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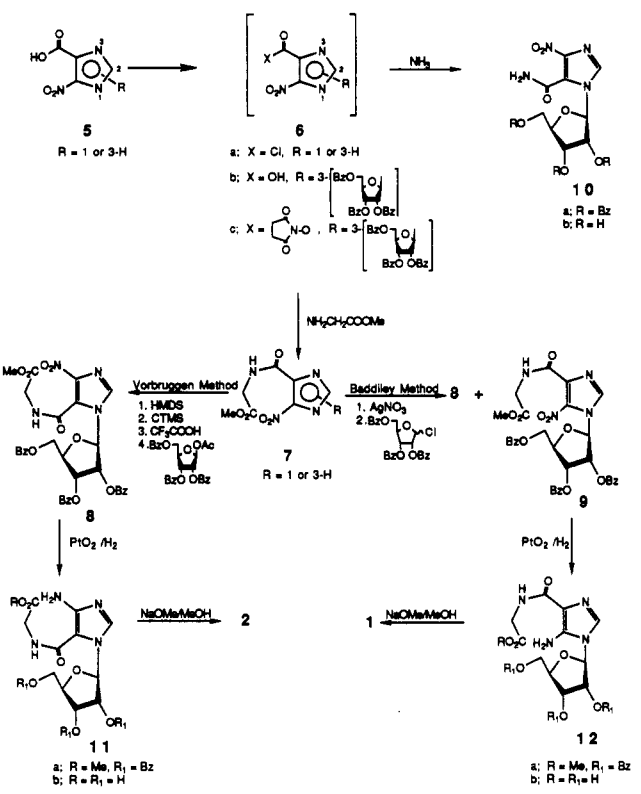
Azepinomycin

examples of 5:7-fused heterocyclic systems include the recently synthesized imidazo[4,5-*e*][1,2,4]triazepine[3g] and

Scheme I



imidazo[4,5-*e*][1,3]diazepine[4f] ring systems. The coformycins possess a tetrahedral geometry at the hydroxyl junction of their seven-membered ring and thus are considered to be transition state analogue inhibitors.[8] The title nucleo(s/t)ides, on the other hand, are structurally more comparable to the natural purine nucleo(s/t)ides, and as such can act as substrates or inhibitors of enzymes of nucleic acid metabolism.

Results and Discussion

The Synthesis of Nucleosides.[3h] Our synthesis (Scheme I) commenced with the reaction of the nitrocarboxyimidazole (**5**)[3a,9,10] with thionyl chloride to obtain **6a**. Treatment of **6a** with glycine methyl ester provided the corresponding amide **7**, which was a suitable precursor for the target nucleosides **1** and **2**. While the Vorbrüggen method[11] of ribosidation of **7** via silylation provided a single nucleoside (**8**), the method of Baddiley[12] via silver salt[13] gave a mixture of two regioisomeric nucleosides **8** and **9** in a ratio of 1.4:1. Although the two isomers should be differentiable by ^{1}H NMR, based upon the anticipated[3e] enhanced deshielding effect of the nitro group on the imidazole H-2 (resonance effect) and the anomeric H-1′ (inductive effect) of **9** as compared with those of **8**, the observed differences in chemical shifts were too small for either the H-2 ($\Delta\delta$ = 0.02) or the H-1′ ($\Delta\delta$ = 0.22) to make

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Table I. CD and UV Spectral Data of Ring-Expanded Nucleosides and Nucleotides, As Contrasted with Those of Xanthosine and Xanthosine 5′-Diphosphate[a]

compound number	UV λ_{max}, nm	ϵ	CD λ_{max}, nm	θ, deg	$[\theta]\lambda_{max}$ (CD), cm^2 deg/mol	$\Delta\epsilon$
xanthosine	262.5	9000	265	-3.1	-1484	-0.45
xanthosine 5′-diphosphate	261	9000	262	-5.7	-2892	-0.87
1	263.5	7100	253	40.3	15292	4.63
2	267.5	7200	268	5.6	5126	1.55
13a	264.5	7100	252	-5.6	-4984	-1.51
14a	268	7200	266	5.0	4854	1.47
13c	254.5	7100	247	-15.70	-8093	-2.45
14c	267.5	7200	267	5.7	5571	1.69

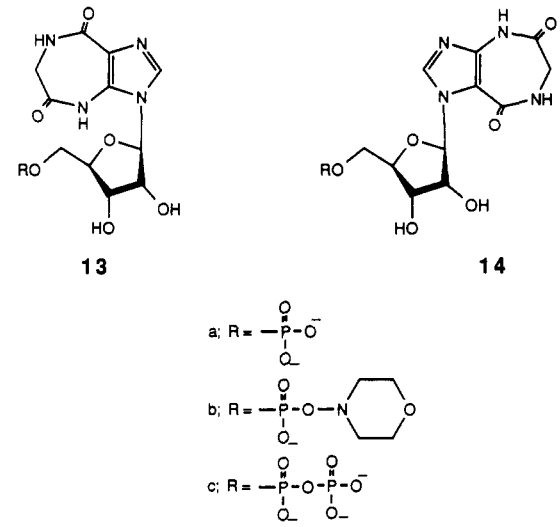
[a] All spectra were obtained in distilled H_2O at 25 °C.

definitive assignments. The small differences may be due to the large deshielding caused by the combined mesomeric and inductive effects of the amide C=O group on the H-2 and H-1′ of **8** as compared with **9**. Likewise, the UV spectra of the two isomers were practically identical. Consequently, the two isomers were distinguished by unequivocal synthesis of **8** from **6c** and glycine methyl ester. The structure of **6c**, which was prepared by sequential Vorbrüggen ribosidation of **5** (to **6b**) and condensation with *N*-hydroxysuccinimide, was confirmed by conversion to the known nucleoside **10b**.[12] The synthesis of the target nucleosides **1** and **2** was completed by sequential reactions involving reduction of **8** and **9** with PtO_2/H_2 to the corresponding amino compounds, **11a** and **12a**, and ring closure with NaOMe/MeOH. This procedure also gave the ring open carboxylic acids **11b** and **12b**, respectively, as side products. The UV spectra of **1** and **2** were very similar in neutral or near neutral pH ($\lambda_{max} \simeq 265$ nm; $\epsilon \simeq 7 \times 10^3$) and both showed considerable bathochromic shifts in basic pH ($\Delta\lambda_{max} \simeq 30$ nm).

Single-Crystal X-ray Analyses of Nucleosides 1 and 2. The structures of **1** and **2** were confirmed by single-crystal X-ray diffraction analyses. The ORTEP views along with the employed atom numbering schemes are shown in Figures 1 and 2, respectively. As anticipated, the seven-membered ring in each is puckered. The two lactam N—C(=O) bonds deviate by about 25 ± 3° from planarity, and the C(C=O) to C(C=O) torsional angle is 75 ± 3°. An important structural difference between **1** and **2** lies in their respective base–ribose conformational relationship: syn in **1** ($\Phi_{C,N}$ = 149°),[15] and anti in **2** ($\Phi_{C,N}$ = 19.9°).[15] Another major distinction between the two regioisomers concerns their sugar pucker: while **1** possesses the $C_{2'\text{-endo}}$–$C_{3'\text{-exo}}$ geometry, that of **2** is the opposite $C_{2'\text{-exo}}$–$C_{3'\text{-endo}}$. Finally, the glycosyl bond length of **1** (1.46 Å) is comparable to that in purine nucleosides, whereas that of **2** (1.49 Å) is closer to the one found in pyrimidine nucleosides.[16]

The syn conformation of **1** is stabilized by intramolecular hydrogen bonding between the 5′-hydroxyl group and the N^4-H of the diazepine ring (5′-O···HN = 1.92 Å). This is unusual since, as a rule, the purine nucleosides, including xanthosine, assume an anti conformation in the crystal lattice[14,15] unless a bulky substituent[16] such as bromine,[17] *tert*-butyl,[18] or an α-hydroxyisopropyl[19] group is attached to the imidazole ring at the C-8 position. Exceptions are also known with nucleoside phosphates (nucleotides)[20,21] and sugars other than ribosides, e.g. arabinosides.[22] Forces leading to the intramolecular hydrogen bonding and the consequent stability of the syn conformation remain to be determined.

Scheme II



The most interesting aspect of the crystal structure of **1** is its molecular packing array in the unit cell. The stereoscopic view reveals an intricate hydrogen bonding and stacking network of 12 identical molecules, vertically placed in two symmetric, helical-looking, coparallel rows, so as to give an overall impression of a multitiered RNA single strand with its bases buried in the sugar backbone, and running in the 2′→5′ direction. While there are several different intermolecular and intertier hydrogen bondings present in the unit cell in addition to the intramolecular 5′-O···N^4–H hydrogen bonding, it appears that the intertier interactions stabilizing the dimer are primarily hydrophobic, and include π-π stacking interactions of the heterocyclic bases in the parallel strands.

The Synthesis of Nucleotides. Nucleosides **1** and **2** were converted into the corresponding nucleotide derivatives (Scheme II), using classical chemical methods of phosphorylation.[23-25] The structures of the mono- and

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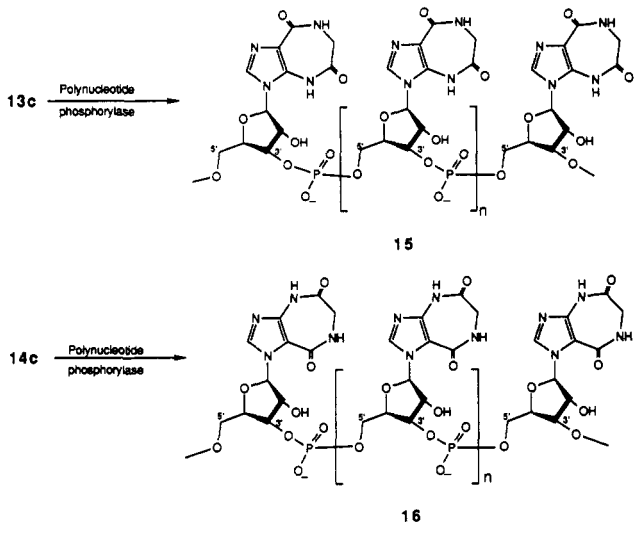
Scheme III



diphosphates were established by UV, ^{1}H NMR, and ^{31}P NMR spectroscopy. The observed values for the ^{31}P chemical shifts correlate well with those reported for *lin*-benzo-AMP and *lin*-benzo-ADP, with 85% H_3PO_4 as reference.[26]

The CD Spectra of Nucleosides and Nucleotides. Conformations in Solution. As indicated by values of *molar ellipticity* [θ] in their respective CD spectra in water (Table I), the nucleoside **1** exhibits a significant change in conformation in going from nucleoside to nucleotides, whereas **2** does not seem to be affected during the same transformation. The marked CD spectral change observed in the transition, **1** → **13a**, may be due either to a parallel change in the syn ⇄ anti conformational equilibrium,[16,27] or as described in a recent paper,[27h] due simply to a large change in glycosyl torsional angle with no effect on the overall conformational equilibrium. It is more likely, however, that the geometric constraint imposed by the phosphate group leads to the loss of the intramolecular hydrogen bond upon conversion of **1** to nucleotide **13a** or **13c**, consequently resulting in an increased population of the anti conformer. By contrast, the conformation of **2**, which is presumably restricted to the anti orientation due to the otherwise severe interactions between the oxygen atoms of the furanose ring (O-4′) and the C-8 carbonyl group, does not alter upon conversion to the corresponding nucleotides, and thus, the [θ] values remain reasonably constant for **2**, **14a**, and **14c**.

The ^{1}H NMR spectrum (DMSO-d_6) of **2** clearly reveals the two NH signals (CH_2N^7-*H* as a triplet at δ 8.06 and a sharp N^4-*H* singlet at δ 10.78). The spectrum of **1**, on the other hand, shows only the N^7-H signal, a triplet at δ 7.82. Further attempts were not, however, made to determine

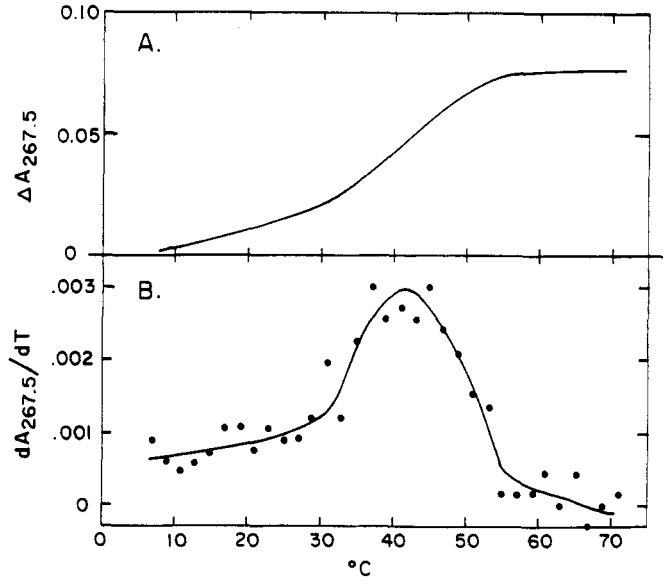


Figure 3. (a) Absorbance–temperature profile of poly[2-MP] (**16**) [5.7×10^{-5} M(p)] in the presence of spermine (5×10^{-5} M). Absorbance was monitored at 267.5 nm. (b) First derivative plot of Figure 3a above.

if the missing N^4-H of **1** was involved in intramolecular hydrogen bonding as in the solid state or was simply exchanging with solvent molecules or with adventitious water ($^3/_4H_2O$) present in the crystals of **1** (see the Experimental Section).

The Synthesis of Homopolymers. The homopolymers poly[1-MP] (**15**) and its isomer poly[2-MP] (**16**) were prepared (Scheme III) by treating the respective diphosphates **13c** and **14c** with the primer-independent polynucleotide phosphorylase from *E. coli* in the presence of Mn^{+2}.[28] The extent of polymerization was determined (see the Experimental Section) by the assessment of (a) the inorganic phosphate release from the polymerization reaction,[29] (b) the ultraviolet absorbance of the extensively dialyzed product, and (c) the lengths of the homopolymers by gel electrophoresis.[30]

The Secondary Structure of Homopolymers. As considerable hyperchromic effect is observed upon increase in temperature, homopolymer **16** appears to possess significant secondary structure. In the low ionic strength ($\simeq$0.01) buffer, there was a small decrease in absorbance when the temperature was lowered from ambient to below 10 °C. This became more obvious when NaCl was added to test solutions. At 0.1 M NaCl, the absorbance–temperature profiles showed a hyperchromic effect of about 12% with an apparent T_m of $\simeq$10 °C. At 0.5 M NaCl, there was a further increase in percent hyperchromicity (17%) and T_m ($\simeq$13 °C), as well as at 1.0 M NaCl (23% and 14.5 °C, respectively). Parallel control experiments with poly(X) reproduced the expected T_ms (27 °C at 0.1 M NaCl; 38 °C at 0.5 M NaCl).[31]

Since the melting temperature of **16** seemed to be strongly dependent on ionic strength, the effect of stoi-

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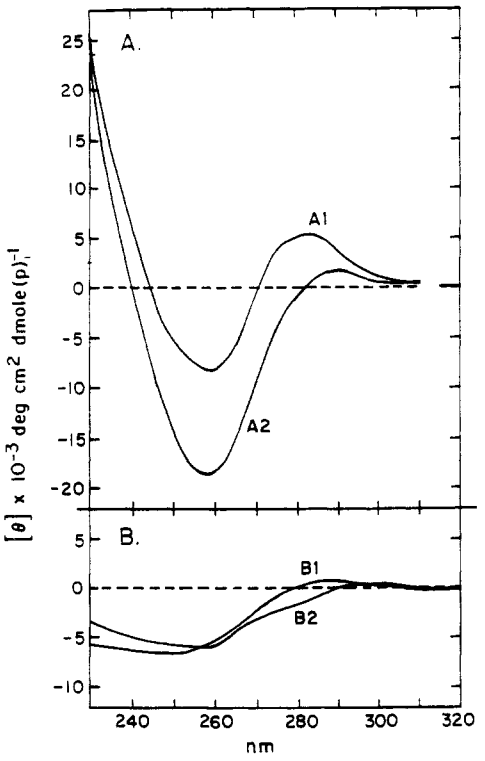

Figure 4. Circular dichroism spectra of poly[1-MP] (**15**) and poly[2-MP] (**16**): A1, 6.6 × 10^{-5} M(p) **16**; A2, 6.2 × 10^{-5} M(p) **16** with 6.0 × 10^{-5} M spermine. B1, 8.9 × 10^{-5} M(p) **15**; B2, 8.5 × 10^{-5} M(p) **15** with 6.0 × 10^{-5} M spermine.

chiometric levels of spermine, a cation with a charge of +4, was assessed. When aliquots of spermine were added to the solutions of **16** in low ionic strength buffer at room temperature or below, there was an immediate hypochromic change. In the presence of stoichiometric levels of spermine, **16** displays a very broad absorbance–temperature profile, with a T_m of $\simeq$ 40 °C and hyperchromicity of $\simeq$ 30–35%, indicating the presence of intrastrand secondary structure (Figure 3). The extent of hyperchromicity is probably maximal, since in the presence of spermine, a significant increase in absorbance occurred only above 10 °C. Parallel melting experiments of poly(U) in the presence of spermine showed the expected abrupt absorbance change at the T_m (26 °C), characteristic of the poly(U)–poly(U) double helix.

The results with homopolymer **15**, by contrast, gave little indication of secondary structure without spermine, and even with spermine the T_m ($\simeq$28 °C) and hyperchromicity (17% or less) were lower than those observed for **16**.

The above findings on secondary structures of **15** and **16** were corroborated by their CD spectra (Figure 4). In low ionic strength buffer, **16** showed a prominant Cotton effect with a maximum at 283 nm, crossover at 271, and a minimum at 260 nm. Upon addition of stoichiometric levels of spermine, there was a significant change in the spectrum with a doubling of the absolute value of the minimum (see Figure 4A), a reduction and a shift of the maximum (to 290 nm), and a shift of the crossover to 282 nm. The perturbation of the CD spectrum correlates well with the hypochromic effect observed upon addition of spermine and is suggestive of a helical structure. By contrast, the CD spectra of **15** is relatively unperturbed by the addition of spermine (Figure 4B), which is in agreement with the small effect of this cation on the UV absorbance–temperature profiles of **15** mentioned above.

Preliminary Biological Screening of 1 and 2. Nucleosides **1** and **2** were screened for activity against murine leukemia virus (MuLV) reverse transcriptase in tissue culture systems.[32] While **1** showed no activity, that of **2** was significant and was comparable to (or slightly better than) that of interferon at the same dosage level. At a dosage of 0.01 μg/mL, **2** rendered 86% protection against MuLV infection. Under the same experimental conditions and dosage level, the cytotoxicity of **2** was also considerably (24%) less than that of interferon.

Conclusions

Ring-expanded nucleoside **1** exhibits an unusual predominantly syn base–ribose conformation both in the solid state and in solution, locked by strong intramolecular hydrogen bonding. It crystallizes in a pseudohelical molecular packing array dictated by a complex network of hydrogen bonding and stacking interactions. The conformation of isomeric **2**, on the other hand, is anti both as a solid and in solution. The sugar pucker geometry in **1** is $C_{2'\text{-endo}}$–$C_{3'\text{-exo}}$, whereas that in **2** is the opposite $C_{2'\text{-exo}}$–$C_{3'\text{-endo}}$. The 5′-diphosphates of both **1** and **2** are substrates for *E. coli* polynucleotide phosphorylase, the yield of the homopolymer being higher with the latter diphosphate. Homopolymer **16** also revealed significant internal secondary structure which was apparently absent in isomeric **15**. While **1** is devoid of antiretroviral activity, **2** is as active as and less toxic than interferon. Whether or not the observed activity of **2** or the inactivity of **1** has any bearing on the correspondingly observed presence or absence of anti base–sugar conformation, $C_{2'\text{-exo}}$–$C_{3'\text{-endo}}$ sugar pucker, helical structure/stability, and the relative ease of enzymic polymerization is yet too premature to conclude. Our contemplated studies on the N^4-methyl analogue of **1**, whose bulky methyl substituent is anticipated to force the base–ribose conformation to an anti orientation, may throw further light on this subject.

Experimental Section

Multiplicity of ^{13}C NMR signals is based on off-resonance ^1H decoupled spectra. Unless stated otherwise, the reported mass spectral fragments are for the EI mode. CI mass spectra were obtained by using either methane or isobutane as the reagent gas. CD spectra were recorded using pathlengths of 1 mm for nucleosides and nucleotides and 1 cm for polynucleotides. X-ray crystal structure analyses were performed at the Department of Chemistry, Southern Methodist University, Dallas, TX. Dry solvents were prepared as follows: methanol, ether, toluene, and xylene were distilled over sodium metal; acetonitrile was distilled from CaH_2, followed by distillation from P_2O_5; DMF and DMSO were distilled at reduced pressure from CaH_2; THF was first dried over KOH and then distilled over sodium. All dry solvents were stored over 3- or 4-Å molecular sieves.

5(4)-Nitro-1(3)*H*-imidazole-4(5)-carboxylic Acid Chloride (6a). In a flame-dried three-neck round-bottom flask, fitted with a guard tube, was placed 5[3a,9,10] (5 g, 31.8 mmol). Thionyl chloride (20 mL, 0.27 mol) was introduced through a serum cap, and the reaction mixture was heated to 50 °C with continuous stirring for 24 h. The compound never went into solution but as the reaction progressed the color became dark yellow. It was rotary evaporated under anhydrous conditions, and the residue was coevaporated with dry toluene three times, when a highly hygroscopic yellow powder of **6a** was obtained. Without further purification, it was employed for the next step.

4-Nitro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-imidazole-5-carboxylic Acid (6b). Prepared from **5**, using the Vorbrüggen procedure of ribosidation[11] as described for compound

(32) Biological screening was carried out by Dr. John Bilello of the Veterans Administration Hospital, Baltimore, MD, and his assitance in this regard is gratefully acknowledged. The murine systems employed for assays include LP-BM5, a mixture of ecotropic and MCF murine leukemia virus (MuLV), and Cas-Br-M.

8 below. It was recrystallized from EtOH–ligroin, yield >90%, mp 197–199 °C: ^{1}H NMR (Me_2SO-d_6) δ 8.03–7.43 (m, 16 H, CH of imidazole + 3 OBz), 6.47–6.46 (d, J_{CH-CH} = 4.0 Hz, 1 H, anomeric CH), 6.10–6.08 (dd, 1 H, ribose CH), 5.93–5.90 (dd, 1 H, ribose H), 4.80–4.70 (m, 3 H, ribose CH and CH_2); IR (KBr) 3778–3482, 1722, 1712, 1618 cm^{-1}; UV (MeOH) λ_{max} 282 nm, 275.5, 229.5.

***N*-Succinimidyl 4-Nitro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)imidazole-5-carboxylate (6c).** A mixture of **6b** (0.5 g, 0.82 mmol), dry THF (80 mL), and dry CH_3CN (20 mL) was stirred, under N_2, at room temperature. Dicyclohexyl carbodiimide (175 mg, 0.84 mmol) was added, and the stirring continued to form a clear solution. *N*-Hydroxysuccinimide (94 mg, 0.82 mmol) was introduced, and the reaction mixture was continued to stir for 2 h. TLC [silica gel, acetone–$CHCl_3$ (1:1)] indicated the formation of a new compound which moved faster than the starting material. The precipitated dicyclohexylurea (DCU) was filtered in vacuo, using anhydrous conditions. The filtrate was equally divided into two portions, stored under anhydrous conditions, and used directly in the next step. A portion of this was used to prepare **8** and the other to prepare **10a**.

5(4)-Nitro-4(5)-(*N*-((methoxycarbonyl)methyl)carbamoyl)-1(3)*H*-imidazole (7). Crude compound **6a** obtained above was placed in a three-neck round-bottom flask, maintained under N_2. Dry CH_3CN (15 mL) was added, followed by the addition of a cold CH_2Cl_2 solution of glycine methyl ester (3 g, 33 mmol), which was freshly liberated from the corresponding hydrochloride salt in 20 mL of CH_2Cl_2 by treatment with triethylamine at 0 °C. The color changed to dark brown immediately after the addition. The reaction mixture was stirred at room temperature for 10 h. Some solid had separated. The mixture was evaporated to dryness on a rotary evaporator, and the residue was dissolved in boiling MeOH (100 mL), treated with decolorizing charcoal, and filtered. Concentration and cooling of the filtrate afforded a solid which was recrystallized from MeOH as off-white shining crystals of **7**, yield 76%, mp 221–223 °C: ^{1}H NMR (Me_2SO-d_6) δ 9.22–9.20 (t, J_{NH-CH_2} = 4.5 Hz, 1 H, NH, exchangeable with D_2O), 7.88 (s, 1 H, imidazole CH); 4.11 (d, J_{CH_2-NH} = 5.5 Hz, 2 H, CH_2), 3.67 (s, 3 H, OMe); IR (KBr), 3318, 1732, 1656, 1510, 1508 cm^{-1}; mass spectrum, *m/e* 228 (M^+), 197, 169,140; UV (MeOH) λ_{max} 303 nm. Anal. Calcd for $C_7H_8N_4O_5$: C, 36.85; H, 3.53; N, 24.56. Found: 36.76; H, 3.55; N, 24.48.

5-(*N*-((Methoxycarbonyl)methyl)carbamoyl)-4-nitro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)imidazole (8). Method A. Ribosidation of 7, Using the Vorbrüggen Procedure.[11] A mixture of **7** (1 g, 4.3 mmol), 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (2.21 g, 4.3 mmol), and freshly distilled dry acetonitrile (30 mL), contained in a dry three-neck flask, maintained under N_2, was stirred at room temperature for 30 min. Freshly distilled 1,1,1,3,3,3-hexamethyldisilazane (HMDS) (1.3 mL, 6 mmol) was introduced through a syringe needle, and the reaction mixture was stirred for 20 min. Chlorotrimethylsilane (CTMS) (0.9 mL, 7 mmol) was introduced when the reaction turned milky, followed by the addition of freshly distilled trifluoromethanesulfonic acid (0.7 mL, 7.9 mmol) when the reaction became clear. The stirring was continued for another $^1/_2$ h. TLC of the reaction mixture [silica gel, toluene–H_2O–acetic acid (5:1:5)] showed complete consumption of the starting material and the formation of a new short-wave UV-absorbing product. The reaction mixture was transferred to a 500-mL beaker, 100 mL of dry CH_2Cl_2 was added, followed by a saturated aqueous solution of $NaHCO_3$. It was stirred for 10 min and transferred to a separating funnel. The organic layer was separated, washed with saturated NaCl solution, dried over anhydrous sodium sulfate for 3 h, and filtered in vacuo, and the filtrate evaporated to dryness. The thick frothy oil obtained was dissolved in boiling EtOH, and when cooled white shining crystals of **8** separated out. It was recrystallized from boiling EtOH to obtain an analytical sample, yield 82%, mp 154–156 °C: ^{1}H NMR (Me_2SO-d_6) δ 9.68–9.58 (t, J_{NH-CH} = 5.6 Hz, 1 H, NH, exchangeable with D_2O), 8.40 (s, 1 H, imidazole CH), 8.10–7.34 (m, 15 H, 3 OBz), 6.46–6.39 (d, J_{CH-CH} = 5.6 Hz, 1 H, anomeric CH), 6.21–5.95 (m, 2 H, ribose CH's), 4.91–4.73 (m, 3 H, ribose CH_2 + CH), 4.13–3.06 (d, J_{CH_2-NH} = 5.6 Hz, 2 H, side-chain CH_2), 3.62 (s, 3 H, OMe); IR (KBr) 3358, 1752, 1732, 1722, 1714, 1708, 1660, 1560, 1460 cm^{-1}; UV (MeOH) λ_{max} 282, 275, 230 nm. Anal. Calcd for $C_{33}H_{28}N_4O_{12}$: C, 58.92; H, 4.16; N, 8.32. Found: C, 58.85; H, 4.22; N, 8.29.

Method B. Reaction of 6c with Glycine Methyl Ester. One of the two portions of the filtrate containing **6c** (vide supra) was placed in a dry 100-mL flask, protected from moisture (N_2), and equipped with a magnetic stirrer. Glycine methyl ester (0.3 g, 3.3 mmol), freshly liberated from the corresponding hydrochloride salt by treatment with triethylamine at 0 °C in CH_2Cl_2, was introduced through a syringe needle. The reaction mixture was stirred at room temperature for 2 h and rotary evaporated to dryness, and the residue was recrystallized two times from EtOH to obtain **8** as a white, fluffy solid, yield 49% (based on **6b**), mp 153–156 °C. The spectral data of this compound were identical with those of **8** prepared by method A above.

Method C. Ribosidation of 7 by the Silver Salt Method.[12] This procedure, which gave a mixture of **8** and **9**, is described below.

4-(*N*-((Methoxycarbonyl)methyl)carbamoyl)-5-nitro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)imidazole (9). (i) Silver Salt of 7. Compound **7** (1.5 g, 6.5 mmol) was dissolved in a mixture of EtOH–H_2O (1:1, 100 mL) by warming in a 500-mL Erlenmeyer flask. A solution of $AgNO_3$ (1.3 g, 7.6 mmol) in 70 mL of hot EtOH was added dropwise to the clear solution of **7** in aqueous EtOH, when a light yellow precipitate was formed. The acidic solution (pH 2) was neutralized with dilute NH_4OH and then boiled in a water bath at 60–65 °C for 2 h. Celite (3.5 g) was added with constant stirring, and the reaction mixture was cooled in a ice water–salt bath. The solid was filtered and washed consecutively with 100 mL each of H_2O, EtOH, and Et_2O. The yellow solid cake was air-dried and then dried in a vacuum oven over P_2O_5 for 96 h. Dry weight of the yellow powder including the Celite was 5.3 g.

(ii) Ribosidation. In a dry three-neck flask, fitted with a distillation set-up and a guard tube (anhydrous $CaCl_2$ + $CaSO_4$), was placed the above Ag salt of **7** mixed with Celite (5.3 g). Dry xylenes (freshly distilled over Na metal) (100 mL) was added, and the reaction mixture was heated to boiling with vigorous stirring. Distillation was continued until 50 mL of xylenes was collected in the receiving flask and discarded. This was done to azeotrope any moisture present in the salt–Celite mixture and to ensure dry reaction conditions. The distillation set was then replaced with a reflux condenser equipped with a guard tube. Freshly prepared 1-chloro-2,3,5-tri-*O*-benzoyl-D-ribofuranose[33] (6.6 mmol) in xylenes (20 mL) was introduced through a syringe needle, and the reaction mixture was refluxed for 4 h. TLC [silica gel, toluene–acetic acid–H_2O (5:5:1)] showed the formation of two distinctly UV-absorbing compounds, the faster moving having the same R_f as **8**. The light brown reaction mixture was cooled and filtered to remove inorganic salts, and the precipitate was washed with excess CH_2Cl_2. The organic filtrate was successively washed with 30% aqueous KI and H_2O and then dried over anhydrous sodium sulfate overnight. Rotary evaporation yielded a viscous yellow oil, which was dissolved in hot EtOH and cooled. On cooling, a crystalline solid separated which was collected by filtration in vacuo and identified as **8** (38%). The EtOH filtrate, as revealed by TLC, contained mainly **9** along with traces of **8**. The filtrate was evaporated to dryness, and the residue was purified by flash chromatography on silica gel (40–63 μm), using a mixture of toluene–EtOAc (8:1) as the eluting solvent. Compound **9** was obtained as a light yellow foam, yield 27%, mp 68–78 °C: ^{1}H NMR (Me_2SO-d_6) δ 8.87–8.84 (t, J_{NH-CH_2} = 6.1 Hz, 1 H, NH, exchangeable with D_2O), 8.42 (s, 1 H, imidazole CH), 8.03–7.30 (m, 15 H, 3 OBz), 6.68–6.64 (d, J_{CH-CH} = 3.2 Hz, 1 H, anomeric CH), 6.15–5.91 (m, 2 H, ribose CH's), 4.90–4.70 (m, 3 H, ribose CH_2 + CH), 4.06–3.98 (d, J_{CH_2-NH} = 6.1 Hz, 2 H, side-chain CH_2), 3.66 (s, 3 H, OMe); IR (KBr) 3400, 1732, 1714, 1632 cm^{-1}; UV (MeOH) λ_{max} 281.5 nm, 274.5, 229.5. Anal. Calcd for $C_{33}H_{28}N_4O_{12}$: C, 58.92; H, 4.16; N, 8.32. Found: C, 58.84; H, 4.22; N, 8.26.

4-Nitro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-imidazole-5-carboxamide (10a). One of the two portions of the filtrate containing **6c**, described above, was placed into a dry two-neck flask, maintained at 0 °C, under anhydrous conditions

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(N_2). Ammonia gas was bubbled into the solution for 3 min, and the reaction mixture was stirred at 0 °C for 1 h. It was rotary evaporated to dryness, the residue was triturated with CH_3CN and filtered to remove the precipitated non-UV-absorbing solid, and the filtrate was once again evaporated to dryness. The residue was purified by flash chromatography on silica gel (40–63 μm), using toluene–EtOAc (8:3) as the eluting solvent. A white solid obtained was recrystallized from EtOH–petroleum ether (40–60 °C) into shiny white crystals of **10a**, yield 73% (based on **6b**), mp 105–108 °C: ^{1}H NMR (Me_2SO-d_6) δ 8.47 (s, 1 H, exchangeable with D_2O, amide NH), 8.30 (s, 1 H, imidazole CH), 8.27 (s, 1 H, exchangeable with D_2O, amide NH), 8.03–7.15 (m, 15 H, 3 OBz), 6.37–6.36 (d, J_{CH-CH} = 5.0 Hz, 1 H, anomeric CH), 6.09–6.07 (dd, 1 H, ribose CH), 5.90–5.88 (dd, 1 H, ribose CH), 4.89–4.88 (m, 1 H, ribose CH), 4.79–4.70 (m, 2 H, ribose CH_2); IR (KBr) 3390, 1732, 1720, 1624 cm^{-1}; UV (MeOH) λ_{max} (MeOH) 281 nm, 275, 230.

4-Nitro-1-β-D-ribofuranosylimidazole-5-carboxamide (10b). Ammonia gas was bubbled for 5 min into a two-neck flask containing dry MeOH (25 mL) at 0 °C. Compound **10a** (150 mg, 0.25 mmol) was added, and the reaction mixture was stirred at room temperature for 24 h. It was evaporated to dryness, and the residue was recrystallized from MeOH into white crystals of **10b**, yield 63%, mp 175–177 °C (lit.[12] mp 175–177 °C): ^{1}H NMR (Me_2SO-d_6) δ 8.38 (s, 1 H, exchangeable with D_2O, amide NH), 8.26 (s, 1 H, imidazole CH), 8.17 (s, 1 H, exchangeable with D_2O, amide NH), 5.63 (s, 1 H, exchangeable with D_2O, OH of ribose), 5.56–5.55 (d, J_{CH-CH} = 5.0 Hz, 1 H, anomeric CH), 5.23–5.22 (d, 1 H, exchangeable with D_2O, OH of ribose), 5.12–5.11 (m, 1 H, exchangeable with D_2O, OH of ribose), 4.28–4.26 (m, 1 H, ribose CH), 4.09–4.07 (m, 1 H, ribose CH), 3.94 (s, 1 H, ribose OH), 3.69–3.56 (m, 2 H, ribose CH_2); IR (KBr) 3445, 1684, 1500 cm^{-1}; UV (H_2O) λ_{max} 295 nm, (pH 0.7) 295, (pH 12.5) 300.5.

4-Amino-5-(*N*-((methoxycarbonyl)methyl)carbamoyl)-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)imidazole (11a). A mixture of compound **8** (1.0 g, 1.4 mmol), absolute MeOH (60 mL), and PtO_2 monohydrate (100 mg) was hydrogenated in a Parr hydrogenator at 40 psi for 45 min. The reaction mixture was filtered twice over Celite, and the filtrate was evaporated to dryness. The residue was triturated with Et_2O, and the precipitated dirty yellow solid was recrystallized from CH_3CN–hexanes to give **11a**, yield 64%, mp 75–78 °C: ^{1}H NMR (Me_2SO-d_6) δ 8.34 (s, 1 H, imidazole CH), 7.99–7.41 (m, 16 H, 3 OBz + NH), 6.73–6.65 (d, J_{CH-CH} = 4.0 Hz, 1 H, anomeric CH), 5.96–5.94 (m, 1 H, ribose CH), 5.84–5.82 (m, 1 H, ribose CH), 5.37–5.35 (br s, 2 H, NH_2, exchangeable with D_2O), 4.75–4.63 (m, 3 H, ribose CH and CH_2), 4.0–3.9 (d, J_{CH_2-NH} = 5.0 Hz, 2 H, side-chain CH_2), 3.65 (s, 3 H, OMe); IR (KBr) 3366, 1718–1740, 1700 cm^{-1}; UV (MeOH) λ_{max} 275 nm, 230, (pH 13) 273.5, (pH 0.8) 273, 230.5. Anal. Calcd for $C_{33}H_{30}N_4O_{10}$·$^1/_4H_2O$: C, 61.68; H, 4.71; N, 8.72. Found: C, 61.25; H, 4.75; N, 8.65.

5-Amino-4-(*N*-((methoxycarbonyl)methyl)carbamoyl)-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)imidazole (12a). Prepared from **9**, using the procedure described above for **11a**, except for the following changes: Evaporation of the alcoholic filtrate yielded a light yellow foam, which was purified by flash chromatography [silica gel (40–63 μm), $CHCl_3$–MeOH (39:1)] into an off-white foam which was crystallized from a minimum volume of 2-propanol, 40–43%, sinters at 85 °C and melts at 93–95 °C: ^{1}H NMR (Me_2SO-d_6) δ 8.0–7.42 (m, 17 H, one H exchangeable with D_2O, N*H*CH_2 + imidazole CH + 3 OBz), 6.30–6.23 (d, 1 H, anomeric CH), 6.10–5.27 (m, 4 H, two H exchangeable with D_2O, NH_2 + two ribose CH's), 4.74 (m, 3 H, ribose CH + CH_2), 3.94–3.87 (d, J_{CH_2-NH} = 5.6 Hz, 2 H, CH_2), 3.61 (s, 3 H, OMe); IR (KBr) 3400, 1730, 1640, 1584 cm^{-1}; UV (MeOH) λ_{max} 267.5 nm, 230, (pH 0.69) 273, 231, (pH 13) 272, 228. Anal. Calcd for $C_{33}H_{30}N_4O_{10}$: C, 61.68; H, 4.71; N, 8.72. Found: C, 62.06; H, 4.98; N, 8.32.

4,5,7,8-Tetrahydro-6*H*-3-β-D-ribofuranosylimidazo[4,5-*e*][1,4]diazepine-5,8-dione (1) and 5-Amino-4-(*N*-(carboxymethyl)carbamoyl)-1-β-D-ribofuranosylimidazole (12b). Dry MeOH (25 mL), which was freshly distilled over sodium, was introduced in a three-neck flask, maintained under a stream of N_2. Freshly cut sodium (100 mg, 4.3 mg-atom) was added with stirring to form a clear solution. Compound **12a** (1.0 g, 1.5 mmol) was added in portions, and the reaction mixture was heated at reflux for 48 h. TLC [silica gel, $CHCl_3$–MeOH (3:2)] indicated the formation of two new UV-absorbing compounds. The reaction mixture was cooled, neutralized with CH_3CO_2H or 1 N HCl, and evaporated to dryness. The residue was purified by flash chromatography on silica gel (40–63 μm), employing (a) $CHCl_3$–MeOH (4:1) to collect the fast eluting **1**, followed by (b) $CHCl_3$–MeOH (1:1) to collect the slower eluting **12b**.

Compound **1** was recrystallized from $CHCl_3$–MeOH as white crystals, yield 52%, mp >170 °C dec: ^{1}H NMR (Me_2SO-d_6) δ 7.87 (s, 1 H, imidazole CH), 7.82 (t, J_{NH-CH_2} = 5.4 Hz, 1 H, NH, exchangeable with D_2O), 5.66–5.65 (d, J_{CH-CH} = 6 Hz, 1 H, anomeric CH), 5.21–5.20 (d, J_{OH-CH} = 4.5 Hz, 1 H, ribose OH, exchangeable with D_2O), 4.23–4.20 (t, J_{CH-CH} = 5.45 Hz, 1 H, ribose CH), 4.06–4.05 (d, J_{OH-CH} = 3.5 Hz, 1 H, ribose OH, exchangeable with D_2O), 4.07–4.04 (dd, J_{OH-CH} = 4.5 and 3.5 Hz, 1 H, ribose OH, exchangeable with D_2O), 3.93–3.91 (dd, J_{CH-CH} = 3 Hz, 1 H, ribose CH), 3.67–3.56 (m, 5 H, ribose (CH + CH_2) + ring CH_2); ^{13}C NMR (Me_2SO-*d6*) δ 168.95 (s, C=O), 164.50 (s, C=O), 134.02 (d, imidazole C-2), 131.89 (s, junctional C next to 7-ring NH), 122.44 (s, junctional C next to 7-ring C=O), 88.06 (d, ribose *C*H), 85.44 (d, ribose *C*H), 74.31 (d, ribose *C*H), 70.01 (d, ribose *C*H), 60.83 (t, ribose CH_2), 45.59 (t, ring CH_2); IR (KBr) 3360–2700 (br), 1690, 1640 cm^{-1}; UV λ_{max} (pH 6.1) 263 nm (ε 7.1 × 10^3), (pH 13.2) 294 (8 × 10^3), 247 (9.2 × 10^3). Anal. Calcd for $C_{11}H_{14}N_4O_6$·$^3/_4H_2O$: C, 42.38; H, 5.01; N, 17.97: Found: C, 42.32; H, 5.02; N, 17.99.

Compound **12b** was isolated as the sodium salt and recrystallized from acetonitrile into a white powder: yield 29%; ^{1}H NMR (Me_2SO-d_6) δ 7.45–7.44 (t, J_{NH-CH_2} = 4.2 Hz, 1 H, NH, exchangeable with D_2O), 7.32 (s, 1 H, imidazole CH), 5.8 (br s, 2 H, NH_2, exchangeable with D_2O), 5.46–5.44 (d, J_{CH-CH} = 4.8 Hz, 1 H, anomeric CH), 5.3–5.15 (br, 3 H, 3 OH, exchangeable with D_2O), 4.27–4.24 (t, 1 H, ribose CH), 4.04–4.02 (m, 1 H, ribose CH), 3.88–3.87 (m, 1 H, ribose CH), 3.71–3.69 (d, J_{CH_2-NH} = 4.2 Hz, 2 H, side-chain CH_2), 3.57–3.56 (m, 2 H, ribose CH_2); IR (KBr) 3400–3100 (br), 1630–1560 (br) cm^{-1}; UV λ_{max} (pH 6.8) 266 (ε 12.9 × 10^3), (pH 12.5) 266.5 (12.8 × 10^3), (pH 0.7) 269.5 (10.2 × 10^3). Anal. Calcd for $C_{11}H_{15}N_4O_7Na$: C, 39.08; H, 4.97; N, 16.08. Found: C, 39.01; H, 4.46: N, 16.56.

4,5,7,8-Tetrahydro-6*H*-1-β-D-ribofuranosylimidazo[4,5-*e*][1,4]diazepine-5,8-dione (2) and 5-Amino-4-(*N*-(carboxymethyl)carbamoyl)-1-β-D-ribofuranosylimidazole (11b). These two compounds were obtained from **11a**, following the procedure described above for **12a** → **1** + **12b**.

Compound **2** was recrystallized from $CHCl_3$–MeOH as shiny white crystals, yield 62%, mp 275–278 °C: ^{1}H NMR (Me_2SO-d_6) δ 10.78 (s, 1 H, NH, exchangeable with D_2O), 8.24 (s, 1 H, imidazole CH), 8.07–8.05 (t, J_{NH-CH_2} = 5.2 Hz, 1 H, NH, exchangeable with D_2O), 6.21–6.20 (d, J_{CH-CH} = 4.0 Hz, 1 H, anomeric CH), 5.43–5.42 (d, J_{OH-CH} = 5.2 Hz, 1 H, ribose OH, exchangeable with D_2O), 5.078–5.065 (d, J_{OH-CH} = 5.2 Hz, 1 H, ribose OH, exchangeable with D_2O), 5.05–5.04 (d, J_{OH-CH} = 5.6 Hz, 1 H, ribose OH, exchangeable with D_2O), 4.10–4.08 (m, 1 H, ribose CH), 4.04–4.03 (m, 1 H, ribose CH), 3.87–3.85 (m, 1 H, ribose CH), 3.71–3.67 (m, 2 H, CH_2), 3.66–3.56 (m, 2 H, CH_2); ^{13}C NMR (Me_2SO-d_6) δ 168.65 (s, C=O), 161.85 (s, C=O), 143.18 (s, junctional C next to 7-ring NH), 137.75 (d, imidazole C-2), 110.82 (s, junctional C next to 7-ring C=O), 89.24 (d, ribose *C*H), 84.20 (d, ribose *C*H), 75.58 (d, ribose *C*H), 68.97 (d, ribose *C*H), 60.24 (t, ribose CH_2), 45.84 (t, ring CH_2); IR (KBr) 3500–3000 (br), 1682, 1632 cm^{-1}; UV λ_{max} (pH 7.2) 267.5 nm (ε 7.2 × 10^3), (pH 12.8) 294 (8 × 10^3), (pH 0.69) 263.5 (7.6 × 10^3). Anal. Calcd for $C_{11}H_{14}N_4O_6$: C, 44.25; H, 4.69; N, 18.77: Found: C, 44.27; H, 4.79; N, 18.73.

Compound **11b** was obtained as a sodium salt and recrystallized as an off-white powder from CH_3CN–MeOH, yield 27%, mp > 180 °C (slow dec): ^{1}H NMR (Me_2SO-d_6) δ 7.72 (s, 1 H, imidazole CH), 7.60–7.58 (t, J_{NH-CH_2} = 5 Hz, 1 H, NH, exchangeable with D_2O), 5.75–5.74 (d, J_{CH-CH} = 5.5 Hz, 1 H, anomeric CH), 5.6–5.4 (br, 2 H, NH_2, exchangeable with D_2O), 5.3–5.0 (br, 3 H, 3 OH, exchangeable with D_2O), 4.15–4.13 (m, 1 H, ribose CH), 4.0–3.9 (m, 1 H, ribose CH), 3.82–3.81 (m, 1 H, ribose CH), 3.78–3.77 (d, J_{CH_2-NH} = 5 Hz, 2 H, side-chain CH_2), 3.66–3.53 (m, 2 H, ribose CH_2); IR (KBr) 3460–3120 (br), 1650, 1635 cm^{-1}; UV (H_2O) λ_{max} 272.5 nm, (pH 12.1) 273, (pH 0.6) 268.5, 245.5. Anal. Calcd for $C_{11}H_{15}N_4O_7Na$·$^1/_4H_2O$: C, 38.50; H, 4.55; N, 16.33. Found: C, 38.47; H, 4.93; N, 16.34.

Disodium 4,5,7,8-Tetrahydro-5,8-dioxo-6*H*-3-β-D-ribofuranosylimidazo[4,5-*e*][1,4]diazepine-5′-phosphate (13a).

Compound 1 (100 mg, 0.3 mmol) was placed in a flame-dried 10-mL round-bottom flask. Freshly distilled trimethyl phosphate (3 mL) was added, and the flask was sealed with a serum cap and immersed in an ice–salt bath. When the temperature reached 0 °C, freshly distilled $POCl_3$ (0.11 mL, 1.18 mmol) was introduced, when a clear solution was formed. The solution was stirred at 0 °C for 2 h. TLC [silica gel, CH_3CN–0.1 M NH_4Cl (7:3)] indicated the complete conversion into a new, slow-moving, short-wave UV-absorbing compound. The reaction mixture was poured over crushed ice (10 g), and the aqueous solution was extracted with ether (3 × 10 mL) to remove excess trimethyl phosphate. The pH of the aqueous solution was further adjusted to 2.5 with 1 N NaOH. The resultant solution was applied to a column of activated carbon (Darco, 12–20 mesh, 30 g). The column was washed with distilled H_2O until the eluate was salt free (tested with $AgNO_3$). It was further eluted with a mixture of EtOH–H_2O–NH_4OH (10:10:1). The UV-absorbing fractions were pooled and evaporated to a small volume which was then passed through an ion-exchange column (Dowex, H^+ form, 50X8-100, 30 g). The column was washed with H_2O until no more UV-absorbing fractions could be detected in the eluate. The appropriate fractions were pooled and concentrated to ≃2 mL. This was passed through a second ion-exchange column (Dowex, Na^+ form, 50X8-100, 30 g). As before, the column was washed with H_2O, UV-absorbing fractions were pooled and rotary evaporated to dryness. The residue was triturated with MeOH, and the separated solid was filtered in vacuo and dried in a vacuum oven to obtain **13a** as an off-white powder, yield 68%: ^1H NMR (D_2O) δ 8.15 (s, 1 H, imidazole CH), 5.74 (s, 1 H, anomeric CH), 4.41 (s, 1 H, ribose CH), 4.32 (s, 1 H, ribose CH), 4.08 (s, 1 H, ribose CH), 3.98, 3.91 (2 s, 4 H, ribose CH_2 + ring CH_2); ^{31}P NMR (D_2O, ref std = α-P of ADP) δ 12.01 (s); IR (KBr) 3500–3000 (br),1700, 1650 cm^{-1}; UV (H_2O) λ_{max} 264 nm, (pH >10) 284, 249.

Disodium 4,5,7,8-Tetrahydro-5,8-dioxo-6*H*-1-β-D-ribofuranosylimidazo[4,5-*e*][1,4]diazepine-5′-phosphate (14a). This compound was prepared from **2** by following the procedure described above for **13a**. It was obtained as a pale yellow solid, yield 70%: ^1H NMR (D_2O) δ 8.38 (s, 1 H, imidazole CH), 6.28 (s, 1 H, anomeric CH), 4.52 (s, 1 H, ribose CH), 4.42 (s, 1 H, ribose CH), 4.28 (s, 1 H, ribose CH), 4.09–4.02 (dd, 2 H, ribose CH_2), 3.94 (s, 2 H, ring CH_2); ^{31}P NMR (D_2O), ref std = α-P of ADP) δ 11.73 (s); IR (KBr) 3700–3000 (br), 1686, 1654 cm^{-1}; UV (H_2O) λ_{max} 267.5 nm, (pH 12.5) 294.5.

Sodium 4,5,7,8-Tetrahydro-5,8-dioxo-6*H*-3-β-D-ribofuranosylimidazo[4,5-*e*][1,4]diazepine-5′-phosphoromorpholidate (13b). The 5′-monophosphate (**13a**) (the free acid obtained from the initial Dowex H^+ ion-exchange column, as described above) (200 mg, 0.52 mmol) was dissolved in a mixture of H_2O (2 mL) and *tert*-butyl alcohol (2 mL), and the solution was transferred to a 50-mL three-neck flask, fitted with a reflux condenser, serum caps, and a magnetic stirrer. To this was added 0.3 mL (3 mmol) of morpholine through a syringe needle. The reaction mixture was heated to reflux. A solution of dicyclohexyl carbodiimide (DCC) (0.6 g, 2.9 mmol) in 3 mL of *tert*-butyl alcohol was added dropwise over a period of 3 h from the top of the condenser (to avoid distillation of morpholine during the addition). The reaction was monitored by TLC [silica gel, isobutyric acid–H_2O–NH_4OH (66:33:1)]. After 15 h, a new UV-absorbing compound was detected, which moved faster than the starting material. The reaction mixture was cooled and filtered to remove 1,3-dicyclohexylurea (DCU), and the filtrate was partially evaporated to remove *tert*-butyl alcohol. The residual DCU was removed by extraction with Et_2O (3 × 10 mL). The aqueous layer was evaporated to dryness using a Kugelrohr apparatus, and the residue was coevaporated with dry pyridine (3 × 10 mL), when a yellow glassy solid was obtained. It was stored in a vacuum oven and was used directly for the next step without further purification.

Sodium 4,5,7,8-Tetrahydro-5,8-dioxo-6*H*-1-β-D-ribofuranosylimidazo[4,5-*e*][1,4]diazepine-5′-phosphoromorpholidate (14b). Prepared using the procedure described above for **13b**.

Trisodium 4,5,7,8-Tetrahydro-5,8-dioxo-6*H*-3-β-D-ribofuranosylimidazo[4,5-*e*][1,4]diazepine-5′-diphosphate (13c).
(A) Preparation of Tri-*n*-butylammonium Phosphate. The cation exchange resin, Dowex 50W-X8-100 (H^+ form, ≃50 g), was washed with 6 N HCl (300 mL) and filtered in vacuo. Washing was repeated until the filtrate was clear. Then it was washed with water (200 mL) and packed into a column as a slurry in H_2O. The column was washed thoroughly with water until the pH of the eluate was neutral. Aqueous pyridine (10%, 150 mL) was run through the column. There was a noticeable color change from dark to light as the column went into the pyridinium form. The pH of the eluate was basic, indicating complete conversion. The column was washed with excess H_2O until the pH of the eluate was neutral. A solution of $NaH_2PO_4 \cdot H_2O$ (0.5 g) in 5 mL of distilled H_2O was passed through the Dowex column (pyridinium form) using H_2O as the eluant. All the UV-absorbing fractions were pooled and evaporated to dryness. A stoichiometric amount (1:1 equiv) of fresh tri-*n*-butylamine (freshly distilled from KOH) was added to the residue, and the mixture stirred for 1 h and then evaporated by azeotroping with dry pyridine (3 × 10 mL). The residue was stored in a vacuum oven until further use.

(B) Reaction with 5′-Phosphoromorpholidate (13b). In a dry 10-mL round-bottom flask, tri-*n*-butylammonium phosphate (0.6 g) was dried by azeotroping with dry pyridine (3 × 5 mL). A solution of dry phosphoromorpholidate (**13b**), prepared as described above, in dry DMF (5 mL) was introduced. The flask was stoppered with a serum cap, and the reaction mixture was stirred at room temperature for 72 h. DMF was removed on a Kugelrohr apparatus equipped with a vacuum pump. The residue was dissolved in 0.05 M triethylammonium bicarbonate (TEAB) buffer (3 mL). The solution was loaded onto a DEAE cellulose column which was preequilibrated successively with 0.5 M TEAB buffer for 24 h, H_2O for 24 h, and finally with 0.05 M buffer for 24 h. The column was eluted with a gradient of TEAB (0.05–0.40 M). The diphosphate was eluted with 0.35–0.4 M buffer. The appropriate fractions were pooled and evaporated to dryness. The residual oily mass was coevaporated to dryness with MeOH (3 × 10 mL). The residue was dissolved in 3 mL of H_2O, and the solution was passed through a column packed with 50 g of Dowex 50X8-100 ion-exchange resin (Na^+ form). The column was eluted with H_2O, and UV-absorbing fractions were pooled and evaporated to dryness when a solid was obtained. It was triturated with MeOH and filtered to obtain **13c** as an off-white solid, yield = 35%: ^1H NMR (D_2O) δ 7.84 (s, 1 H, imidazole CH), 5.62–5.61 (d, J_{CH-CH} = 4 Hz, 1 H, anomeric CH), 4.42–4.41 (d, J_{CH-CH} = 4.5 Hz, 1 H, ribose CH), 4.38–4.37 (d, J_{CH-CH} = 4.0 Hz, 1 H, ribose CH), 4.12 (s, 1 H, ribose CH), 4.05–4.03 (dd, 1 H, CH of 5′-CH_2), 3.96–3.94 (dd, 1 H, CH of 5′-CH_2), 3.62 (s, 2 H, ring CH_2); ^{31}P NMR (D_2O, ref std = α-P of ADP) δ 0.38 (d, $^2J_{P-O-P}$ = 18.1 Hz, α-P), 4.68 (d, $^2J_{P-O-P}$ = 18.0 Hz, β-P); UV (H_2O) λ_{max} (pH 7) 263 sh, 254.5, (pH 12.5) 292.5, 247.5 nm.

Trisodium 4,5,7,8-Tetrahydro-5,8-dioxo-6*H*-1-β-D-ribofuranosylimidazo[4,5-*e*][1,4]diazepine-5′-diphosphate (14c). Prepared from **14b** according to the procedure described above for **13c**. It was obtained as a white solid, yield 38%: ^1H NMR (D_2O) δ 8.30 (s, 1 H, imidazole CH), 6.29–6.28 (d, J_{CH-CH} = 2 Hz, 1 H, anomeric CH), 4.51–4.49 (m, 2 H, two ribose CHs), 4.29–4.28 (m, 1 H, ribose CH), 4.26 (s, 2 H, ribose CH_2), 3.9 (s, 2 H, ring CH_2); ^{31}P NMR (D_2O, ref std = α-P of ADP) δ 0.24 (d, $^2J_{P-O-P}$ = 25.0 Hz, α-P), 4.48 (d, $^2J_{P-O-P}$ = 25.0 Hz, β-P); UV (H_2O) λ_{max} 267.5 nm, (pH 12.2) 295.5 nm.

Poly[1-MP] (15) and Poly[2-MP] (16). These were prepared[28] from the respective nucleoside 5′-diphosphate, **13c** and **14c**, using the primer-independent *E. coli* polynucleotide phosphorylase (Sigma). As a control, parallel polymerization experiments were performed with xanthosine 5′-diphosphate. Generally, a polymerization mixture of 50 μL consisted of 20 mM nucleoside diphosphate, 0.1 M Tris·HCl (pH 9.0), 7 mM $MnCl_2$, 0.01 M β-mercaptoethanol, and 0.2 units of PNPase (4 units/mL final concentration). This mixture was then incubated at 37 °C for periods up to 65 h. The mixture was diluted with an equal volume of 1:3 v/v isoamyl alcohol/chloroform. After slow gentle shaking by hand, the mixture was spun in a microfuge for 2 min, and the aqueous layer containing the polynucleotide was collected by pipetting and resubjected to the above extraction process for a total of three or four times. The extracted polynucleotide was then exhaustively dialyzed vs successive 1000-mL changes (consisting of 2 × 500 mL changes each) of (i) 0.5 M NaCl, 10^{-4} M EDTA, 0.01 M Tris·HCl (pH 7.6), (ii) 0.5 M NaCl, 0.01 M Tris·HCl (pH 7.6), (iii) 0.1 M NaCl, 0.01 M Tris·HCl (pH 7.6), (iv) 0.01 M

NaCl, 0.01 M Tris·HCl (pH 7.6). The molecular weight cut off of the dialysis membrane was 12000–14000 Da.

The extent of polymerization was determined by the following methods: (a) Colorimetric assessment of the release of inorganic phosphate from the polymerization reaction (prior to extraction), which is equimolar to the nucleoside diphosphate reacted, was made at 820 nm by reaction with 0.25% ammonium molybdate in 1% ascorbic acid, 1 N H_2SO_4, using KH_2PO_4 as a standard.[29] The data indicated that about 60% of **13c** reacted to form **15**, comparable to the extent of reaction obtained with XDP or ADP. (b) The ultraviolet absorption spectrum of the dialyzed product, and the number of absorbance units at λ_{max}, were compared with those of the starting material. The yield of nondialyzable, UV-absorbing material (based on A_{265} units) ranged between 7 and 12% for **15** and 15 and 20% for **16**, assuming the same extinction coefficient for polynucleotide residue as for mononucleotides. (c) The lengths of **15** and **16** were estimated by their electrophoretic mobilities on denaturing polyacrylamide gels containing 11.4% acrylamide, 0.6% bisacrylamide, 28.8% urea in TBE buffer (0.05 M Tris, 0.05 M boric acid, and 0.001 M Na_2EDTA). After electrophoresis, the gels were stained with ethidium bromide. Gel electrophoresis indicated that the material was clearly polymeric, electrophoresing as a broad band whose mobility was comparable to poly(xanthylic acid), and was less than the xylene cyanol dye marker, which runs comparably to a 55-mer of poly(A).[30] Relative to poly(X), **16** was stained poorly by ethidium bromide, and **15** was not visualizable by this method.

The polymerization process and the extraction procedures employed had no adverse effect on the integrity of the polymeric material as indicated by (a) the qualitative absorbance spectrum of the homopolymer which was identical to that of the monomer, except for the possible hyperchromic effect, and (b) the TLC mobility [silica gel, $CHCl_3$–MeOH (3:2)] of the product of digestion of homopolymer **16** with ribonuclease A, snake venom phosphodiesterase, and alkaline phosphatase,[28d] which was indistinguishable from that of the corresponding nucleoside monomer **2**.

Absorbance–Temperature Profiles. Teflon-stoppered micro quartz cuvettes containing 100 μL of test solutions were placed in a Gilford 2400-2 spectrophotometer designed to raise the temperature at a constant rate, which was 25 °C/h in these experiments. Temperature was continually monitored by means of a calibrated thermistor (Yellow Springs Instruments) inserted through a narrow hole in the stopper of the reference cuvette. Absorbance was usually monitored at the polynucleotide's wavelength of maximum absorbance. The buffer in all experiments was 0.01 M phosphate/Na^+, 0.1 mM EDTA, pH 7.0, with additions of NaCl or spermine as indicated. In order to assure attainment of equilibrium, homopolymer–spermine solutions to be melted were allowed to incubate overnight at 4 °C prior to determination of Tm.

Single-Crystal X-ray Diffraction Analyses of Compounds 1 and 2. Suitable crystals were grown through slow crystallization from the appropriate solvents (see pertinent experimental data above). The unit cell dimensions were obtained by a least-squares fit of 25 centered reflections in the range of $10° < 2\theta < 25°$. Intensity data were collected by using a $\theta/2\theta$ scan type in the range of $3° < 2\theta < 55°$. Three standard reflections monitored after every 100 reflections did not show any significant change in intensity during data collections. Intensities were corrected for decay and Lorentz polarization effects but not for absorption. The structure was solved and all non-hydrogen atoms were found by using results of SHELXTL-PLUS.[34] After several cycles of refinements using SHELX76/SHELXTL-PLUS[34,35] the positions of hydrogen atoms were located on difference Fourier maps, and included in the final refinement with isotropic thermal parameters, and with geometrical constraints for CH_2 and CH protons. Refinement proceeded to converge by minimizing the function $\sum w(|F_o| - |F_c|)^2$, where the weight, w, is $\sigma(F)^{-2}$. The discrepancy indices $R = \sum||F_o| - |F_c||/\sum|F_o|$, and $R_w = [\sum w(|F_o| - |F_c|)^2/\sum w(|F_o|)^2]^{1/2}$ are presented below.

Crystallographic Data. A. Compound 1: $C_{11}H_{14}N_4O_6$, M_r = 298.29, space group $P2_12_12_1$, orthorhombic, a = 5.6226 (13) Å, b = 13.956 (5) Å, c = 15.803 (9) Å, V = 1240 (9) Å^3, Z = 4, D_x = 1.60 g cm^{-3}, (Mo Kα) = 0.710 73 Å, μ = 0.12 mm^{-1}. Final R = 3.83%, R_w = 3.94% for 1162 unique reflections with $I \geq 3\sigma(I)$ = 1108.

B. Compound 2: $C_{11}H_{14}N_4O_6$, M_r = 298.29, space group $P2_1$, monoclinic, a = 5.043 (2) Å, b = 7.315 (3) Å, c = 16.825 (6) Å, β = 94.93 (3)°, V = 618.3 (4) Å^3, Z = 2, D_x = 1.60 g cm^{-3}, (Mo Kα) = 0.710 73 Å, μ = 0.12 mm^{-1}. Final R = 3.03%, R_w = 4.35% for 1547 unique reflections with $I \geq 3\sigma(I)$ = 1444.

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Supplementary Material Available: Tables of bond lengths, bond angles, torsional angles, and positional parameters for compounds **1** and **2** (12 pages). Ordering information is given on any current masthead page.

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