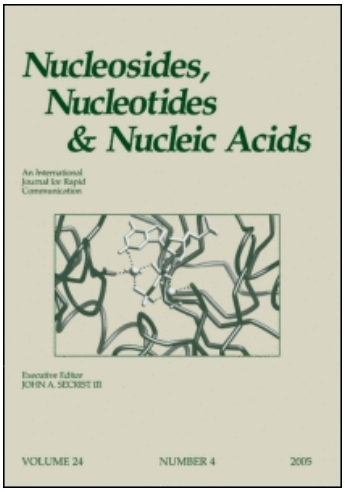

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The Synthesis of Novel Regioisomeric Ring-Expanded Xanthine Nucleosides Containing The 5:7-Fused Imidazo[4,5-e][1,2,4]Triazepine Ring System

Vishweshwar S. Bhadti[a]; Anila Bhan[a]; Ramachandra S. Hosmane[a]; Martin Hulce[b]
[a] Laboratory for Chemical Dynamics Department of Chemistry, Biochemistry University of Maryland Baltimore County Baltimore, Maryland [b] Department of Chemistry, Creighton University Omaha, Nebraska

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THE SYNTHESIS OF NOVEL REGIOISOMERIC RING-EXPANDED XANTHINE NUCLEOSIDES CONTAINING THE 5:7-FUSED IMIDAZO[4,5-e][1,2,4]TRIAZEPINE RING SYSTEM

Vishweshwar S. Bhadti, Anila Bhan, and Ramachandra S. Hosmane*

Laboratory for Chemical Dynamics
Department of Chemistry and Biochemistry
University of Maryland Baltimore County
Baltimore, Maryland 21228

and

Martin Hulce*

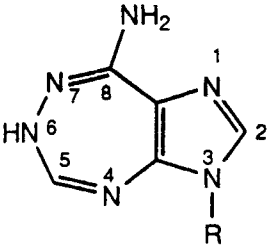
Department of Chemistry
Creighton University
Omaha, Nebraska 68178

ABSTRACT: The syntheses of novel regioisomeric ring-expanded purine nucleosides containing the imidazo[4,5-e][1,2,4]triazepine nucleus are reported. The glycosylation of the heterocycle 3,4,6,7-tetrahydro-imidazo[4,5-e][1,2,4]triazepine-5,8-dione (**2a**) by the stannic chloride procedure gave nucleosides **3** and **4**, with the sugar moiety attached at the 7- and 3-positions of the heterocycle, respectively. On the other hand, the mercuric cyanide procedure for glycosylation of **2a** yielded nucleosides **4** and **5**, with the sugar attached at the 1-position in the latter. In either procedure, **4** was the minor isomer and was obtained only in trace amounts. While debenzoylation of **3** and **5** provided the respective parent nucleosides **8** and **10**, that of **4** resulted in ring-opening to produce **9**. Attempted enzymic glycosylation of **2a** with purine nucleoside phosphorylase failed to yield any nucleoside product.

We have been involved in the synthesis and biochemical/biophysical investigations of a series of ring-expanded ("fat") purine nucleosides and nucleotides, which are of biochemical, biophysical, medicinal, as well as chemical interest.[1] Biochemically, these molecules

potentially are an abundant source of substrates or inhibitors of enzymes of purine metabolism and of those requiring energy cofactors, ATP or GTP. Biophysically, they are excellent probes for steric and conformational constraints of the nucleic acid double helix.[1b] From a medicinal standpoint, they can be regarded as analogues of the well-studied benzo-di/tri-azepines, a family of powerful pharmaceuticals acting on the central nervous system.[2] They are also of interest from a strictly chemical standpoint. Studies relating to their synthesis, structure, acid-base properties, aromaticity, and tautomer equilibria are potentially interesting and rewarding.

We report here the synthesis of another new class of ring-expanded nucleosides which contain the imidazo[4,5-e][1,2,4]triazepine nucleus. Our initial synthetic attempts in this series were directed at an adenine analogue 1, which, however, led to the isolation of rearranged products.[3] While the xanthine analogues 2 were discovered to be considerably more stable, their synthesis nevertheless proved to be far from trivial.[4] The presence of 8-π electrons in the seven-membered ring renders 2 antiaromatic by Hückel standards (4n + 2 rule) and therefore, prone to opportunistic rearrangements.[3] In view of a number of alleged seven-membered and larger ring heterocycles which were later



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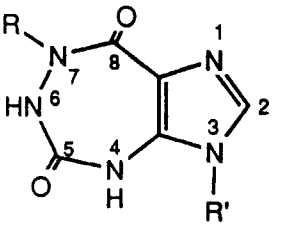


2

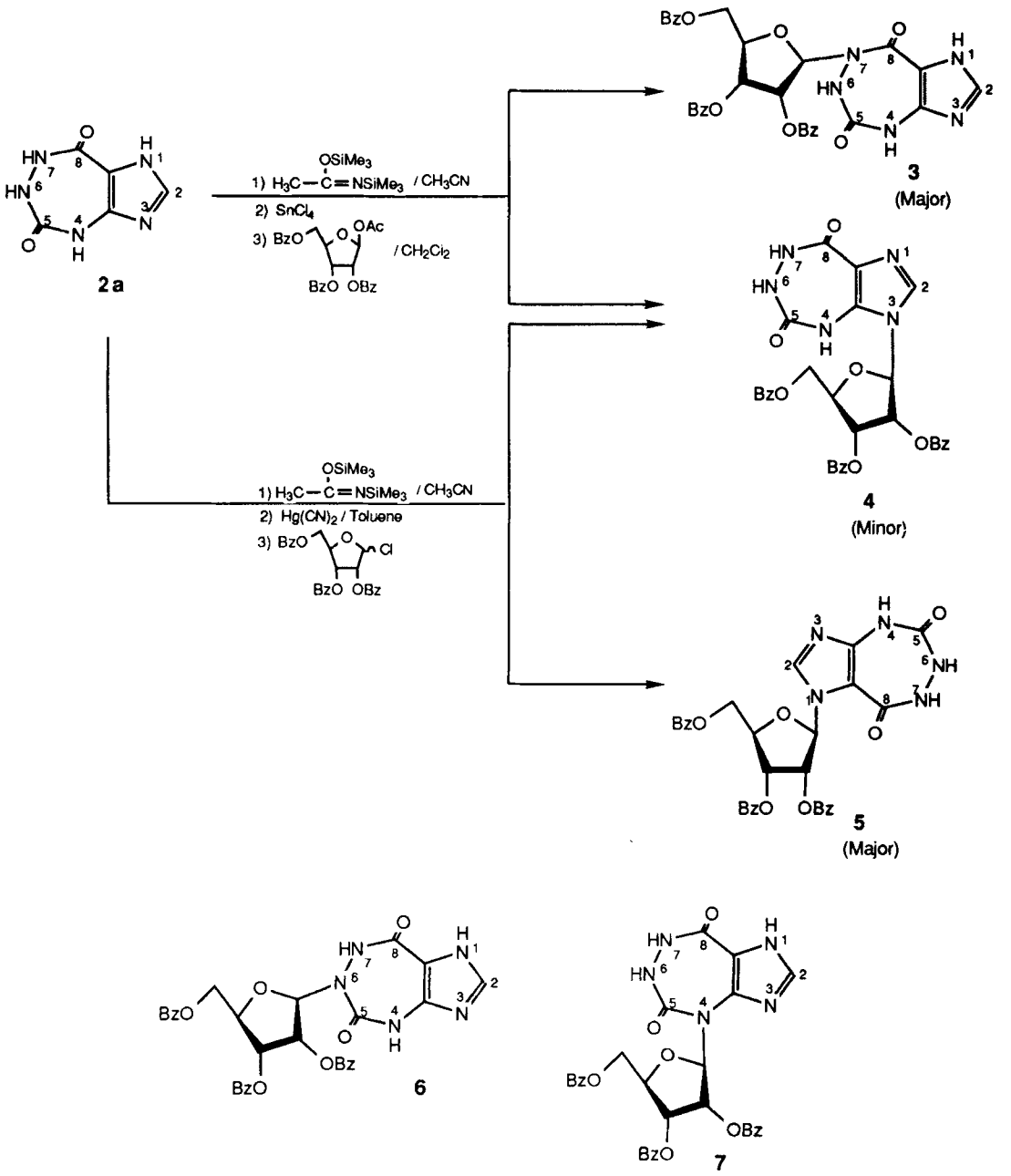
a; R = R' = H
b; R = Me, R' = H
c; R = Me, R' = CH_2Ph
d; R = R' = CH_2Ph
e; R = CH_2Ph, R' = H

proved to be 5- or 6-membered ring systems,[5] structure confirmation of a representative member of this class of compounds by X-ray was especially warranted. The 5:7-fused imidazotriazepinedione skeleton of 2 was indeed confirmed by X-ray diffraction analysis of 2c.[4]

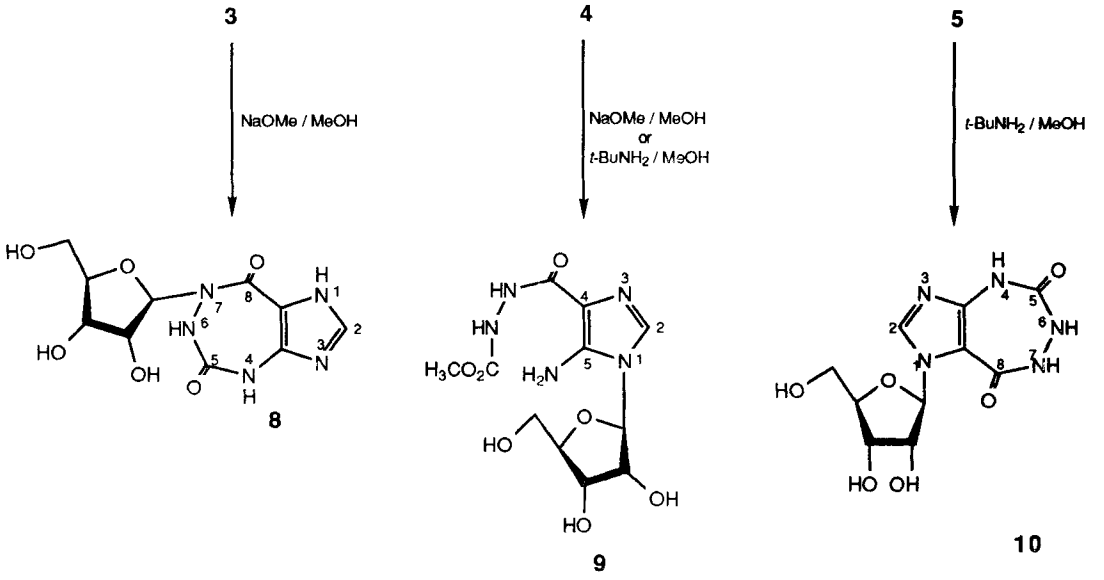
Of seven different procedures attempted for glycosylation of the heterocycle 3,4,6,7-tetrahydroimidazo[4,5-e][1,2,4]triazepine-5,8-dione (**2a**),[4] only two were fruitful, those using stannic chloride[6] or mercuric cyanide[7] as catalysts. The two methods, however, yielded two different results, each giving two regioisomeric products with a common minor isomer. Thus, persilylation of **2a**[4] with N,O-bis(trimethylsilyl)trifluoroacetamide or N,O-bis(trimethylsilyl)acetamide, followed by stannic chloride-catalyzed[6] condensation with 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose, gave two ribosides, **3** (39%) and **4** (traces) (**Scheme I**). In contrast, condensation of persilylated **2a** with 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride, catalyzed by mercuric cyanide,[7] provided ribosides **5** (39%) and **4** (4%).

Regioisomers **3**, **4**, and **5** were distinguished from each other and from the remaining two possible isomers **6** and **7** (**Scheme I**), employing high field (500 MHz) NMR and UV spectroscopy. Compounds **4** and **5** could be readily distinguished from **3**, **6**, and **7** by ^1H-^1H correlation spectroscopy (COSY). The H-6 signal in the COSY of **4** and **5** exhibited a diagnostic double coupling, absent in **3**, **6**, or **7**, which was correlated to H-4 and H-7. In addition, the ^1H NMR spectra of both **4** and **5** revealed the absence of an imidazole H-1 in the δ 12-13 region (the parent heterocycle **2a** exhibits this proton at δ 12.8).[4] This was also corroborated by the ^1H-^1H COSY of **4** and **5**, whose H-2 showed no coupling with H-1, unlike **3**.

Nucleoside **3** was distinguished from **6** and **7** by comparison of the UV spectra of the OH-deprotected **3** (*i.e.* Compound **8**) with those of **2e**.[4] The UV spectra of **8** in pH 7 (λ_{max} 208, 264 nm), pH 12 (215, 290), and pH 2 (229, 255) were closely comparable with those of **2e** at pH 7 (205, 258), pH 13 (212.5, 288.5), and pH 0.5 (228.5, 250.5), including similar ε values for each set of λ_{max} values. Furthermore, the ^1H NMR spectra of both **3** and **2e**[4] exhibited a 4-bond coupling between H-4 (δ ≈ 9.8-10.2 ppm) and H-6 (δ ≈ 8.4-8.8), which was confirmed by their respective ^1H-^1H COSY. Additional evidence for the distinction of **3** from **7** was furnished by the presence of H-4 resonance in the ^1H NMR spectrum of **3** in the δ 10.0 region (the parent heterocycle **2a**[4] exhibits this resonance at δ 9.7). The H-4, being conjugated to two electron-withdrawing C-5 and C-8 carbonyl groups, is anticipated to be more acidic and therefore, more deshielded than either the H-6 or H-7, each



SCHEME I



SCHEME II

of which is conjugated to a single carbonyl group. The N^6 and N^7 protons in **2a** appear in the < δ 9.0 region.[4] The presence of a 4-bond coupling between H-4 and H-6 in the COSY of the product is consistent with structure **3**, and rules out structure **6**.

Distinction between **4** and **5** was based upon the anticipated (see below) and observed lower field ^{1}H NMR signal for H-2 of **5** (δ 8.18) as compared with that of **4** (< δ 8.0, buried in the benzenoid signals). The electron-withdrawing C-8 carbonyl functionality of **5** causes its H-2 to be deshielded relative to the H-2 of **4**, which experiences the electron-donating effects of an N-H group at position 4. The C-2 signals in the ^{13}C NMR spectra of **4** and **5** exhibited a similar pattern. While the C-2 signal in **4** appeared at δ 132.97, that in **5** appeared at 139.01. We have recently observed a similar effect in the analogous regioisomeric ring-expanded nucleosides containing the imidazo[4,5-e][1,4]diazepine skeleton.[1b]

Deprotection of the sugar hydroxyl groups (**Scheme II**) of **3** and **5** with sodium methoxide/methanol and tert-butylamine/methanol, respectively, afforded the corresponding parent nucleosides **8** and **10**.

However, attempts to deprotect **4** using either sodium methoxide or tert-butylamine with methanol as solvent gave exclusively the ring-open product **9**. There was no debenzoylation of **4** with tert-butylamine when methanol was replaced by a non-nucleophilic solvent (THF). The poor yield of **4** from **2a** precluded further attempts of deprotection. The structure **9** was established by ^{1}H NMR, UV, and mass spectral data, coupled with elemental microanalyses. The ^{1}H NMR spectrum of **9** indicated the presence of an amino group at δ 6.01, exchangeable with D_2O, and a methoxy group at δ 3.57.

In view of multiple nucleoside products obtained by chemical methods of glycosylation, it was of interest to see if an enzymic glycosylation of **2a** would produce a single regio- and stereoisomer. In this respect, purine nucleoside phosphorylase (PNP, EC 2.4.2.1) was a logical choice for carrying out enzymic glycosylation. PNP catalyzes the reversible degradation of a wide variety of nucleosides, and has been extensively employed in recent years to synthesize various ^{15}N- and radiolabeled nucleosides[8] from the corresponding heterocyclic bases. However, as most of the reported studies are limited to 5:6-fused ring systems, an a priori assessment of PNP acceptance of the 5:7-fused **2a** could not be made. Employing a literature procedure,[8b] **2a** was subjected to enzymic glycosylation with a bacterial PNP (Sigma). As a control, glycosylation of adenine to adenosine was monitored under identical conditions. The products were analyzed by HPLC. While the conversion of adenine to adenosine could be detected, there was no trace of any of the nucleoside products of **2a** in the chromatogram. It follows, therefore, that **2a** is not a substrate for the employed PNP.

EXPERIMENTAL SECTION

^{1}H and ^{13}C NMR spectra were recorded on an IBM NR/80, GE QE-300 or a GE GN-500 instrument. The reported spectral data are relative to Me_4Si as an internal reference standard unless otherwise indicated. Multiplicity is designated by the abbreviation, s = singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, m = multiplet, br = broad, and app = apparent. Mass spectra were recorded on a Hewlett Packard 5988A mass spectrometer. Elemental microanalyses were performed by Atlantic Microlab, Inc., Norcross, Georgia. Infrared

spectra were obtained on a Perkin-Elmer ratio recording instrument. Ultraviolet spectra were recorded on a Gilford Response UV/VIS spectrophotometer. Melting points are uncorrected. Dry solvents were prepared as follows: 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.

7-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)-1,4,6,7-tetrahydro-imidazo[4,5-e][1,2,4]triazepine-5,8-dione (3). Glycosylation Catalyzed by Tin (IV) Chloride. A mixture of **2a**[4] (140 mg, 0.83 mmol) and N,O-bis(trimethylsilyl)acetamide (1.27 mL, 5.0 mmol) in dry CH_3CN (15 mL) was stirred at room temperature for 12 h, by which time a clear solution had formed. The solvent was evaporated under reduced pressure and the residue was co-evaporated with dry toluene (3 x 5 mL). The residue was dried *in vacuo* and dissolved in CH_3CN (25 mL). The solution was cooled to -29 °C and treated with tin (IV) chloride (0.32 g, 1.25 mmol). The mixture was stirred for 10 min and treated with a solution of 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (0.42 g, 0.83 mmol) in dry CH_2Cl_2 (10 mL). The reaction mixture was gradually allowed to come to room temperature and was stirred for a further period of 1 h. The reaction mixture was poured into a mixture of AcOEt (50 mL) and saturated $NaHCO_3$ solution and stirred. The mixture was filtered through Celite™, the organic layer was separated, and the aqueous layer was extracted with AcOEt. The combined organic layer was dried and concentrated under vacuum to obtain a foamy residue whose TLC [silica gel, $CHCl_3$:MeOH (10:1)] indicated a mixture of **3** (Rf ≈ 0.7) and **4** (Rf ≈ 0.3). The foam was purified by flash chromatography on a silica gel column by gradient elution with $CHCl_3$:MeOH (40:1 → 15:1). Appropriate fractions were combined and concentrated to yield **3** as a foam (0.2 g, 39%): ^1H NMR (DMSO-d_6) δ 13.05 (br s, 1 H, NH, exchangeable with D_2O, H-1), 10.21 (br s, 1 H, NH, exchangeable with D_2O, H-4), 8.81 (br s, 1 H, NH, exchangeable with D_2O, H-6), 8.00-7.39 (m, 16 H, Ar-H + H-2), 6.12 (d, J = 5.0 Hz, 1 H, H-1′), 5.82-5.79 (m, 1 H, H-2′), 5.74-5.72 (m, 1 H, H-3′), 4.67-4.62 (m, 3 H, H-4′ + two H-5′); the ^1H-^1H COSY of **3** indicated strong correlations between H-4 and

H-6 and between H-1 and H-2; ^{13}C NMR (DMSO-d_6) δ 165.99 (>C=O), 164.90 (>C=O), 163.91 (>C=O), 161.61 (>C=O), 147.04 (>C=O), 138.64 (C-2), 133.91 (Ar-C), 133.85 (>C=), 129.42-128 (Ar-C), 109.33 (>C=), 87.52 (C-1'), 77.65 (C-2'), 71.21 (C-3'), 70.81 (C-4'), 64.42 (C-5'); IR (KBr) 3500 (br), 1720 (C=O), 1660 (C=O), 1650 (C=O) cm^{-1}; UV λ_{max} (pH 7) 228 nm (log ε 4.56), 273 (log ε 4.02), 282 sh (log ε 3.93), (pH 13) 223 (log ε 4.57), 272.5 (log ε 3.88), 281 (log ε 3.87), 292 sh (log ε 3.78), (pH 0.5) 228.5 (log ε 4.56), 262 (log ε 3.99).

Anal. Calcd for $C_{31}H_{25}N_5O_9 \cdot \frac{1}{2}H_2O$: C, 59.99; H, 4.22; N, 11.28. Found: C, 60.26; H, 4.20; N, 11.19.

Appropriate fractions from the remaining eluate were pooled and evaporated to obtain traces (< 2%) of **4**. For spectral and physical data on this compound, refer to the mercuric cyanide procedure of glycosylation described below.

3-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)-3,4,6,7-tetrahydroimidazo[4,5-e][1,2,4]triazepine-5,8-dione (4) and 1-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)-1,4,6,7-tetrahydroimidazo[4,5-e][1,2,4]triazepine-5,8-dione (5). Glycosylation Catalyzed by Mercuric Cyanide. A mixture of **2a**[4] (660 mg, 3.95 mmol) and N,O-bis(trimethylsilyl)-acetamide (6.0 mL, 24.2 mmol) in dry CH_3CN (25 mL) was stirred at room temperature for 12 h, by which time a clear solution had formed. The solvent was evaporated under reduced pressure and the residue was co-evaporated with dry toluene (3 x 10 mL). The residue was dried *in vacuo*, dissolved in dry toluene (50 mL), and the solution was treated with $Hg(CN)_2$ (2.0 g, 7.95 mmol). The mixture was heated at 90-95 °C, and treated with a toluene solution (10 mL) of 2,3,5-tri-O-benzoyl-β-D-ribofuranosyl chloride, prepared[9] from 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (2.0 g, 3.96 mmol). The mixture was heated to reflux for 2-3 h, cooled, and the solvent was removed under reduced pressure. The residue was diluted with CH_2Cl_2 (100 mL), filtered through Celite™, and the filtrate was washed with a 30% KI solution (3 x 50 mL), followed by a saturated solution of NaCl (3 x 50 mL). The organic layer was dried and concentrated. The residue, the TLC [silica gel, $CHCl_3$:MeOH (10:1)] of which indicated a mixture of **4** (Rf ≃ 0.3) and **5** (Rf ≃ 0.7), was purified by flash chromatography on a silica gel column with gradient elution, using $CHCl_3$:MeOH (40:1 → 15:1).

Appropriate fractions were pooled and evaporated to collect the higher Rf product **5** as a foam (0.95 g, 39%): ^1H NMR (DMSO-d_6) δ 9.96

(br s, 1 H, NH, exchangeable with D_2O, H-4), 9.39 (br s, 1 H, NH, exchangeable with D_2O, H-7), 8.53 (br s, 1 H, NH, exchangeable with D_2O, H-6), 8.18 (s, 1 H, H-2), 7.99-7.40 (m, 15 H, Ar-H), 6.59 (d, $\underline{J}$ = 4.5 Hz, 1 H, H-1'), 6.07-5.90 (m, 1 H, H-2'), 5.89 (m, 1 H, H-3'), 4.80 (m, 1 H, H-4'), 4.72-4.60 (m, 2 H, H-5'); the ^1H-^1H COSY of **5** showed correlations of H-6 to both H-4 and and H-7, and the high resolution (500 MHz) spectrum of **5** exhibited H-6 as a doublet of doublets; ^{13}C NMR (DMSO-$\underline{d}_6$) δ 165.82 (>C=O), 164.92 (>C=O), 164.76 (>C=O), 164.08 (>C=O), 163.31 (>C=O), 147.69 (>C=), 109.36 (>C=), 139.01 (=CH-, C-2), 134.23 (=CH-), 134.18 (=CH-), 133.88 (=CH-), 129.73 (=CH-), 129.63 (=CH-), 129.5 (>C=), 129.10 (=CH-), 129.08 (=CH-), 128.85 (>C=), 128.77 (>C=), 88.31 (>CH-), 79.30 (>CH-), 74.93 (>CH-), 70.73 (>CH-), 63.95 ($>CH_2$); IR (KBr) 3500-3200 (NH), 1725 (C=O), 1670 (C=O), 1650 (C=O) cm^{-1}; UV λ_{max} (pH 7) 230 nm (log ε 4.62), 263 sh (log ε 4.02), 271 sh (log ε 3.99), 281 sh (log ε 3.83), (pH 13) 227.5 br (log ε 4.62), 271 sh (log ε 3.78), 278.5 (log ε 3.69), 301.5 sh (log ε 3.3), (pH 0.5) 231 (log ε 4.62), 261 sh (log ε 4.02), 272.5 sh (log ε 3.96), 281.5 sh (log ε 3.80).

Anal. Calcd for $C_{31}H_{25}N_5O_9$: C, 60.88; H, 4.12; N, 11.45. Found: C, 60.82; H, 4.14; N, 11.41.

Appropriate fractions from the remaining eluate were pooled and evaporated to obtain **4** as a foam (100 mg, 4%): ^1H NMR (DMSO-$\underline{d}_6$) δ 10.05 (br s, 1 H, NH, exchangeable with D_2O, H-4), 9.19 (br s, 1 H, NH, exchangeable with D_2O, H-7), 8.60 (br s, 1 H, NH, exchangeable with D_2O, H-6), 8.02-7.40 (m, 16 H, Ar-H + H-2), 6.44 (d, $\underline{J}$ = 6.0 Hz, 1 H, H-1'), 5.99 (m, 1H, H-2'), 5.95 (m, 1 H, H-3'), 4.80-4.60 (m, 3 H, H-4' + H-5'); the ^1H-^1H COSY of **4** exhibited correlations H-6 to both H-4 and H-7; ^{13}C NMR (DMSO-$\underline{d}_6$) δ 165.99 (>C=O), 165.80 (>C=O), 164.98 (>C=O), 164.76 (>C=O), 162.96 (>C=O), 135.76 (>C=), 134.30 (=CH-), 134.34 (=CH-), 133.98 (=CH-), 132.97 (=CH-, C-2), 129.76 (=CH-), 129.65 (=CH-), 129.50 (>C=), 129.18 (=CH-), 129.13 (=CH-), 129.05 (=CH-), 128.91 (>C=), 128.54 (>C=), 120.58 (>C=), 84.45 (>CH-), 80.05 (>CH-), 74.35 (>CH-), 71.23 (>CH-), 64.14 ($>CH_2$); IR (KBr) 1720 (C=O), 1660 (C=O), 1650 (C=O) cm^{-1}; UV λ_{max} (pH 7) 231.5 nm (log ε 4.66), 274 sh (log ε 3.85), 280 (log ε 3.76), (pH 13) 218.5 (log ε 4.74), 252.5 (log ε 4.17), 297 sh (log ε 3.5), 301 sh (log ε 3.49), (pH 0.5) 232 (log ε 4.64), 274 sh (log ε 3.80).

Anal. Calcd $C_{31}H_{25}N_5O_9 \cdot \frac{1}{2}H_2O$: C, 59.99; H, 4.22; N, 11.28. Found: C, 60.13; H, 4.15; N, 11.20.

1,4,6,7-Tetrahydro-7-(β-D-ribofuranosyl)imidazo[4,5-e][1,2,4]-triazepine-5,8-dione (8). To a suspension of 3 (300 mg, 0.49 mmol) in dry MeOH (20 mL) was added a solution of NaOMe, freshly prepared by dissolving Na metal (60 mg, 2.61 mg.atom) in dry MeOH (10 mL), at 0-5 °C. The reaction mixture was stirred for 2 h at the same temperature, and then at room temperature for 1 h. The mixture was neutralized with solid CO_2, and the solvent was removed under reduced pressure. The residue was triturated with Et_2O and filtered to obtain a solid which was purified by dissolving in MeOH, and passing the solution through a charcoal column (Darco^R, 12-20 mesh), eluting with a mixture of EtOH:H_2O:NH_4OH (10:10:1). The solvent was evaporated and the residue was further purified by flash chromatography on a silica gel column, using a gradient of $CHCl_3$:MeOH (10:1 → 10:3). Appropriate fractions were pooled and evaporated to obtain a solid which was recrystallized from MeOH-CH_3CN to obtain **8** as a white powder (80 mg, 54.5%), mp 205 °C dec: ^1H NMR (DMSO-d_6) δ 13.0 (s, 1 H, NH, exchangeable with D_2O, H-1), 10.18 (s, 1 H, NH, exchangeable with D_2O, H-4), 8.21 (s, 1 H, NH, exchangeable with D_2O, H-4), 7.71 (s, 1 H, H-2), 5.65 (d, J = 5.0 Hz, 1 H, H-1′), 5.33 (m, 1 H, OH, exchangeable with D_2O), 5.15 (m, 1 H, OH, exchangeable with D_2O), 5.06 (m, 1 H, OH, exchangeable with D_2O), 4.08-3.98 (m, 2 H, H-2′ and H-3′), 3.77 (m, 1 H, H-4′), 3.60-3.52 (m, 2 H, H-5′); IR (KBr) 3500-3240 (br), 1700 (C=O), 1670 (C=O) cm^{-1}; UV λ_{max} (H_2O) 208 nm (log ε 4.12), 266 (log ε 3.63), (pH 13) 209 (log ε 4.65), 295 (log ε 3.56), (pH 1) 216 (log ε 3.88), 255 (log ε 3.70).

Anal. Calcd for $C_{10}H_{13}N_5O_6 \cdot \frac{1}{2}H_2O$: C, 38.96; H, 4.58; N, 22.72. Found: C, 38.91; H, 4.53; N, 22.62.

N^1-Methoxycarbonyl-N^2-(5-amino-1-β-D-ribofuranosyl-4-imidazolylcarbonyl)hydrazine (9). A suspension of **4** (0.2 g, 0.32 mmol) in dry MeOH (30 mL) was treated with tert-butylamine (0.2 mL, 1.9 mmol) while cooling at 0 °C. The mixture was allowed to come to room temperature and then was stirred for 4 h. The solvent was evaporated and the residue was thoroughly washed successively with Et_2O and CH_2Cl_2. The crude product was purified by flash chromatography on silica gel by gradient elution with a mixture of $CHCl_3$-MeOH (10:1 → 10:3). Appropriate fractions were pooled and evaporated, and the residual

solid was recrystallized from EtOH to obtain **9** as colorless crystals (80 mg, 75.5%), mp 213-215 °C: ^{1}H NMR (DMSO-d_6) δ 9.15 (s, 1 H, NH, exchangeable with D_2O), 8.87 (s, 1 H, NH, exchangeable with D_2O), 7.35 (s, 1 H, H-2), 6.01 (s, 2 H, NH_2, exchangeable with D_2O), 5.48 (d, J =5.5 Hz, 1 H, H-1'), 5.41 (br s, 1 H, OH, exchangeable with D_2O), 5.29 (br s, 1 H, OH, exchangeable with D_2O), 5.18 (d, J = 2.5 Hz, 1 H, OH, exchangeable with D_2O), 4.27 (m, 1 H, H-2'), 4.03 (m, 1 H, H-3'), 3.85 (m, 3 H, H-4' + two H-5'), 3.57 (s, 3 H, OMe); IR (KBr) 3460-3100 (br), 1710 (C=O), 1650 (C=O) cm^{-1}; MS (EI, 70 eV) m/z 331 (M^+), 285, 242, 199, 167, 152, 126, 110; UV λ_{max} (MeOH) 271 nm, (pH 13) 215, 275, (pH 1) 209, 247, 272.

Anal. Calcd for $C_{11}H_{17}N_5O_7$: C, 39.88; H, 5.17; N, 21.14. Found: C, 39.92; H, 5.19; N, 21.10.

1,4,6,7-Tetrahydro-1-(β-D-ribofuranosyl)imidazo[4,5-e][1,2,4]-triazepine-5,8-dione (10). A mixture of **5** (250 mg, 0.41 mmol) and *t*-butylamine (200 mg, 2.7 mmol) in dry MeOH (20 mL) was stirred at 0 °C for 2 h. The solvent was removed under reduced pressure, and the residue was washed with CH_2Cl_2. The solid obtained was recrystallized from 2-propanol to obtain **10** as a white powder (90 mg, 73%), mp 228-230 °C: ^{1}H NMR (DMSO-d_6) δ 9.80 (br s, 1 H, NH, exchangeable with D_2O, H-4), 9.29 (br s, 1 H, NH, exchangeable with D_2O, H-7), 8.46 (br s, 1 H, NH, exchangeable with D_2O, H-6), 8.13 (s, 1 H, H-2), 6.09 (d, J = 3.0 Hz, 1 H, H-1'), 5.45 (d, J = 5.5 Hz, 1 H, OH, exchangeable with D_2O), 5.09-5.05 (m, 2 H, OH, exchangeable with D_2O), 4.11 (m, 1 H, H-2'), 4.02 (m, 1 H, H-3'), 3.86 (m, 1 H, H-4'), 3.64-3.53 (m, 2 H, H-5'); IR (KBr) 3500-3100 (br), 1710 (C=O), 1650 (C=O) cm^{-1}; UV λ_{max} (H_2O) 208 nm (log ε 4.24), 254 (log ε 3.82), (pH 13) 247 (log ε 4.0), 291.5 (log ε 3.26), (pH 0.5) 227 (log ε 3.97), 251 (log ε 3.86).

Anal. Calcd for $C_{10}H_{13}N_5O_6 \cdot 0.2\ (CH_3)_2CHOH$: C, 40.90; H, 4.72; N, 22.49. Found: C, 40.97; H, 4.61; N, 22.46; Anal. (HRMS, FAB) Calcd for $C_{10}H_{14}O_6N_5$ (MH^+) m/z 300.0943. Observed 300.0944.

Attempted Enzymic Glycosylation of 2a with Purine Nucleoside Phosphorylase. A mixture (total volume 285 μL) containing **2a** (88 μM) or adenine (88 μM, used as a control), Tris-HCl (pH 7.4, 175 mM), dithiothreitol (DTT, 8.8 mM), α-D-ribose-1-phosphate or 2-deoxy-α-D-ribose-1-phosphate (877 μM), bovine serum albumin (219 μg/mL), and PNP

(bacterial, Sigma, 1.75 μg /mL), was incubated at 37 °C for 1.5 h. The reaction mixture was analyzed by an analytical HPLC (ISCO), using a C18 column (5 x 250 mm, ISCO) and water as an eluting solvent (flow rate 1 mL/min). The effluent was monitored at 254 nm. In the control reaction, both adenine (retention time = 11.5 min) and adenosine (r.t. = 6.5 min) were detected on the chromatogram [eluent = H_2O-MeOH (85:15), flow rate = 1.5 mL/min], but only **2a** (r.t. = 7 min) was present in the sample reaction mixture.

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