42-7; **10**, 50585-43-8; **11**, 50585-44-9; **12**, 50585-45-0; **13**, 50585-46-1; catechol dipotassium salt, 50585-47-2; 1,2,4,5-tetrafluorobenzene, 327-54-8; 1,2,3,4-tetrachlorodibenzo-*p*-dioxin, 30746-58-8; hexachlorobenzene, 118-74-1; 1,2,3,4-tetrachlorobenzene, 634-66-2; pentachlorobenzene, 608-93-5; 4,5-dimethylcatechol dipotassium salt, 50585-48-3; 2,3,7-trichlorodibenzo-*p*-dioxin, 33857-28-2; 4-chlorocatechol dipotassium salt, 50585-49-4; 4,5-dichlorocatechol dipotassium salt, 50585-50-7.

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Synthesis of Some Tricyclic Nucleosides Related to the "Y" Base of tRNA[1a,b]

Gary L. Anderson,[1c] Boshra H. Rizkalla, and Arthur D. Broom*

Department of Biopharmaceutical Sciences, College of Pharmacy, University of Utah, Salt Lake City, Utah 84112

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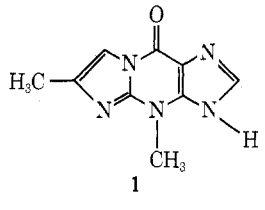
The synthesis of three tricyclic nucleosides, 5*H*(7*H*)-9-oxo-3-β-D-ribofuranosyl-1,2,4-triazolo[2,3-*a*]purine (**3**), 6,7-dimethyl-10-oxo-3-β-D-ribofuranosyl-1,2,4-triazino[2,3-*a*]purine (**4**), and 10-oxo-3-β-D-ribofuranosyl-1,2,4-triazino[2,3-*a*]purine (**5**), is reported. These are structural analogs of the "Y" base of tRNA. The use of the nuclear Overhauser effect in proton assignment of **3** is described, as well as the fluorescence of **3** and **4**. Covalent hydration of **5** is discussed.

Transfer ribonucleic acids specific for phenylalanine ($tRNA^{Phe}$) from a variety of sources have recently been shown to contain certain highly fluorescent heterocyclic bases, the simplest of which is the tricyclic derivative **1**.[2] Other tricyclic fluorescent derivatives of naturally occurring purines, exemplified by 1,N^6-ethenoadenosine (ε-adenosine), have recently been synthesized[3] and shown to enter into a number of biochemical pathways.[4] The availability of 1-aminoguanosine (**2**) in our laboratories[5] led us to undertake the synthesis of certain tricyclic nucleosides derived from guanosine and structurally related to the "Y" base (**1**).



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Results and Discussion

Synthetic Aspects. The cyclization procedures used to obtain the tricyclic nucleosides are shown in Scheme I. Attempts to prepare the triazolopurine ribonucleoside **3** using diethoxymethyl acetate[6] gave complex mixtures from which **3** could be isolated only with great difficulty. The procedure of Clark and Lister[7] using DMF-$POCl_3$ has been widely used for cyclization of weakly basic 1,2-diamino compounds. Application of this procedure to 1-aminoguanosine (**2**) gave the desired **3** in good yield. The structure of **3** was confirmed by elemental analysis and uv and pmr spectra. The uv spectra (Table I) reveal substantial bathochromic shifts relative to starting material **2** and are very similar to those reported for the imidazo[1,2-*a*]purine ribonucleoside obtained by the reaction of guanosine with glycidaldehyde.[8]

The condensation of 1,2-diaminopyrimidines with 1,2-dicarbonyl compounds (the Isay synthesis) has found extensive use in the synthesis of pteridines.[9] Application of this reaction with **2** using biacetyl and glyoxal gave 6,7-dimethyl-10-oxo-3-β-D-ribofuranosyl-1,2,4-triazino[2,3-*a*]purine (**4**) and its unmethylated derivative **5**, respectively. Nucleoside **4** was found to have elemental analysis and uv and pmr spectra consistent with the assigned structure (Scheme I). The uv spectra of **5**, however, were grossly different from those of its dimethyl counterpart **4** (Table I), the pmr spectra were incompatible with structure **5** (Table II), and elemental analysis revealed the presence of 2.5 equiv of water/mol of **5**. The data were consistent with the existence in solution of **5** as a covalent hydrate and **4** as the anhydrous molecule. The interpretation receives support from the observation by Clark[10] that ethyl pteridine-4-carboxylate readily forms a covalent hydrate in solution whereas its 6,7-dimethyl derivative is only slightly hydrated at equilibrium, and is confirmed by the pmr data discussed below.

Pmr Considerations. Examination of the aromatic region of the pmr spectrum of the triazolo[2,3-*a*]purine nucleoside **3** revealed two one-proton singlets downfield 0.30 and 0.80 ppm from the H-8 signal of 1-aminoguanosine (**2**)

Table I
Uv Absorption Maxima (nm)

Compd	pH 1 λmax	pH 1 Log εmax	pH 7 λmax	pH 7 Log εmax	pH 11 λmax	pH 11 Log εmax
2	257	4.064	255	4.167	257	4.155
3	276	4.068	285	4.163	285	4.153
4	248	4.290	254	4.291	254	4.279
	292	3.634	297	3.606	297	3.629
	358	3.480	362	3.521	362	3.510
5	257	4.029	247	4.145	257	4.093
	295	3.687	255	4.093	306	3.587
			303	3.598		

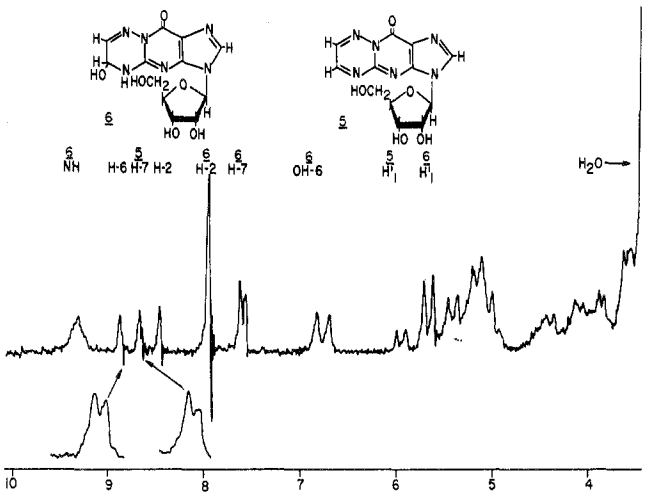


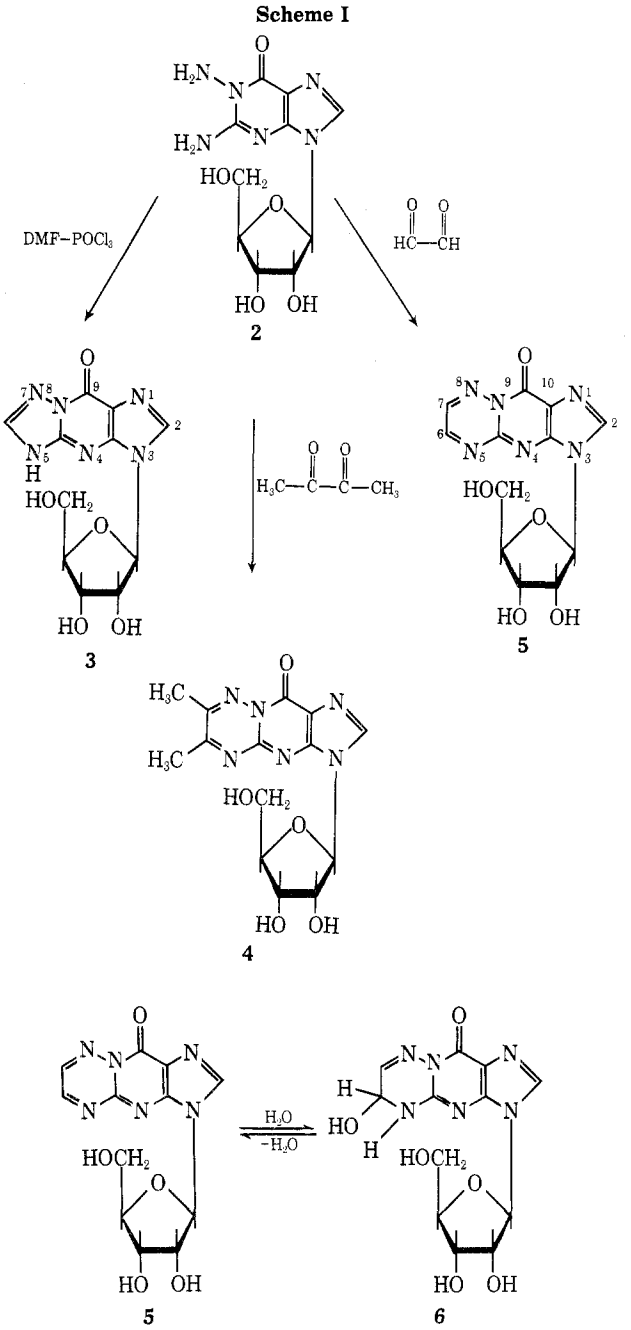
Figure 1. Pmr spectrum in $(CD_3)_2SO$ of 10-oxo-3-β-D-ribofuranosyl-1,2,4-triazino[2,3-*a*]purine (**5**) and its covalent hydrate (**6**). For details of interpretation see text.

(Table II). The H-8 proton of a purine nucleoside is known to undergo facile exchange upon heating in D_2O.[11] Attempts to assign the base protons of **3** using this method failed because the two carbon-bound protons showed similar rates of exchange. Nuclear Overhauser enhancement of pmr signals of specific protons arising from saturation of the spin of a proton in close physical proximity has been used in studies of conformation of purine and pyrimidine nucleosides.[12] Application of this technique permitted a facile assignment of the H-2 and H-6 signals of **3**. Irradiation of the H-1′ signal led to a 14% enhancement of the signal at δ 8.10, whereas the signal at δ 8.60 showed an enhancement of only 5%. Examination of molecular models reveals that, regardless of conformation, H-1′ must be in closer proximity to H-2 than H-6. This permits the assignment of the enhanced signal at δ 8.10 to H-2 and that of the nearly unchanged signal at δ 8.60 to H-6.

The pmr data for **4** (Table II) are also in complete accord with the proposed structure. The H-2 proton of **4** in $(CD_3)_2SO$ solution appears as a sharp singlet at δ 8.36. The remarkable consistency of successive downfield shifts of the imidazole proton and the sugar H-1′ in the series **4** ≅ **5** > **3** > **2** is worthy of note (Table II). The marked deshielding effect on protons distant from the actual site of ring closure may be attributed to an increase in the ring current of the molecule and suggests further that fusion of a six-membered ring (compounds **4** and **5**) to a purine enhances the imidazole ring current to a greater extent than fusion of a five-membered ring (compound **3**).

The pmr spectrum of **5** in $(CD_3)_2SO$ was quite complex and suggestive of the presence of two compounds in solution. In D_2O solution, however, a greatly simplified spectrum satisfying the requirements for a single covalent monohydrate of **5** (compound **6**) was obtained. The salient

Scheme I



features of the pmr spectrum of **6** (Table II) include a sharp singlet for H-2 (δ 7.91), a pair of doublets at δ 7.64 (H-7, "sp^2" type proton) and 5.41 (H-6, methine proton), and a doublet at δ 5.79 corresponding to H-1′. The spectrum in $(CD_3)_2SO$ (Figure 1), in addition to signals corresponding to those described above, contains an additional signal and coupling from the 6-OH proton and a broad signal from the 5-NH. A new set of signals corresponding to the anhydrous compound **5** is observed, including a singlet at δ 8.46 (H-2), a doublet at δ 5.96 (H-1′), and a pair of doublets ($J_{6,7}$ = 1.4 Hz) at δ 8.67 and 8.85 corresponding to the two triazine protons. Unequivocal assignment of the proton signals to the respective molecules was made possible by comparison of the integral values; typically, the ratio of **6** to **5** in $(CD_3)_2SO$ solution was 7:2. The position of hydration was shown by the pmr data cited above to occur across a C=N bond in the triazine ring; the assignment of the 5,6 double bond was made by analogy with recent work in which the 1,2,4-triazine system was shown to undergo covalent hydration at the analogous 4,5 double bond.[13]

Table II
Pmr Frequencies, δ, in $(CD_3)_2SO$ (DSS)

Compd	H_2[a]	H_1'	H_8	H_7	Others
2	7.80 (s)	5.62 (d) $J_{1',2'}$ = 5.7 Hz			6.79 (b) NH_2
3	8.10 (s)	5.77 (d) $J_{1',2'}$ = 5.4 Hz	8.60 (s)		
4	8.36 (s)	5.90 (d) $J_{1',2'}$ = 5.3 Hz			2.59 CH_3 (s) 2.65 CH_3 (s)
4[b]	8.19 (s)	5.90 (d) $J_{1',2'}$ = 5.1 Hz			2.66 CH_3(s) 2.71 CH_3 (s)
5	8.46 (s)	5.96 (d) $J_{1',2'}$ = 5.6 Hz	8.67 (d) $J_{6,7}$ = 1.4 Hz	8.85 (d)	
6	7.94 (s)	5.69 (d) $J_{1',2'}$ = 5.6 Hz	~5.4 (m)[c]	7.59 (d) $J_{6,7}$ = 2.7 Hz	6.77 (d) 6-OH $J_{6H,6OH}$ = 8 Hz 9.22 (b) 5-NH
6[d]	7.91 (s)	5.79 (d) $J_{1',2'}$ = 5.6 Hz	5.41 (d) $J_{6,7}$ = 3.0 Hz	7.64 (d)	

[a] H_8 of purine listed under H_2 for comparison. [b] Solvent D_2O. [c] Obscured by sugar OH protons. [d] Solvent D_2O–$(CD_3)_2SO$ (4:1 v/v).

Fluorescence Studies. A detailed examination of the fluorescence properties of these molecules was not undertaken. Examination of the emission maxima of aqueous solutions of **2**, **3**, and **4**, however, revealed that concentrations of 3×10^{-5} *M* gave measurable emissions at 450 nm with an exciting wavelength of 285 nm for **3** and 540 nm (exciting wavelength 360 nm) for **4** compared with an emission at 370 nm (excitation 285 nm) for **2**. The emission maxima observed for **3** and **4** are far from those observed for purines in general,[14] and are reminiscent of those of the "Y" base.[15]

Experimental Section

Elemental analyses were performed by Heterocyclic Chemical Corp., Harrisonville, Mo. Melting points were taken on a Thomas-Hoover apparatus and are uncorrected. Uv spectra were obtained using a Cary Model 15 spectrometer. Uncorrected fluorescent spectra were obtained using an Aminco-Bowman spectrofluorometer. Pmr spectra were obtained with Jeolco C6OH spectrometer at ambient temperature. NOE experiments were run in frequency sweep mode. H-6 and H-2 peak areas were measured using the integrator when H_1' was decoupled and undecoupled. Each reported area was the average of ten measurements.

5*H*(7*H*)-9-Oxo-3-β-D-ribofuranosyl-1,2,4-triazolo[2,3-*a*]purine (3). 1-Aminoguanosine[5] (**2**, 500 mg, 1.68 mmol), 10 ml of DMF, and 0.5 ml of $POCl_3$ were stirred at room temperature for 3 hr. The white precipitate was filtered, then dissolved in 20 ml of hot EtOH–H_2O (9:1), filtered, and cooled at 5° overnight to give 345 mg (67%) of white crystals. Recrystallization twice from hot water gave an analytical sample, mp 300°.

Anal. Calcd for $C_{11}H_{12}N_6O_5 \cdot 0.5H_2O$: C, 41.6; H, 4.12; N, 26.5. Found: C, 41.5 H, 3.91; N, 26.4.

6,7-Dimethyl-10-oxo-3-β-D-ribofuranosyl-1,2,4-triazino[2,3-*a*]purine (4). To a suspension of **2** (750 mg, 2.5 mmol) in 300 ml of 70% aqueous ethanol was added 2,3-butanedione (1.5 ml, 15 mmol) and 3.75 ml of 0.1 *N* HCl. The suspension was stirred for 10 days at room temperature (a yellow solution resulted after 1 day). The solution was evaporated *in vacuo* and coevaporated with three 100-ml portions of ethanol. The yellow crystals were recrystallized from ethanol to give 537 mg (60%), mp 169–171° dec.

Anal. Calcd for $C_{14}H_{16}N_6O_5 \cdot 0.5H_2O$: C, 47.1; H, 4.79; N, 23.5. Found: C, 47.1; H, 5.00; N, 23.5.

5,6-Dihydro-6-hydroxy-10-oxo-3-β-D-ribofuranosyl-1,2,4-triazino[2,3-*a*]purine (6). To a solution of glyoxal, prepared by refluxing glyoxal trimer (500 mg, 2.9 mmol) in 100 ml of H_2O for 30 min, was slowly added **2** (500 mg, 1.68 mmol) in 100 ml of hot H_2O. The solution was stirred at 50° for 1 hr, then placed on a column of Dowex IR-120 (H^+ form, 100 ml). The column was washed with 2 l. of water. The resin was placed in a beaker and adjusted to pH 6 with NH_4HCO_3 solution, filtered, and washed twice with H_2O and the yellow filtrate was evaporated *in vacuo* to dryness (bath at 30°). The yellow residue was dissolved in 200 ml of H_2O and lyophilized to give 370 mg (60%), mp 180° dec, softens at 110°.

Anal. Calcd for $C_{12}H_{14}N_6O_6 \cdot 1.5H_2O$: C, 39.5; H, 4.69; H, 23.0. Found: C, 39.5; H, 4.75; N, 22.7.

Registry No.—**2**, 19039-33-9; **3**, 50585-21-2; **4**, 50585-22-3; **5**, 50585-23-4; **6**, 50585-24-5.

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