Synthesis and NMR Studies of Some Imidazo[4,5-*d*]pyridazine Nucleosides

R. Paul Gagnier, Michael J. Halat and Brian A. Otter*

Laboratory of Organic Chemistry, Memorial Sloan-Kettering Cancer Center, Sloan-Kettering Division, Graduate School of Medical Sciences, Cornell University, New York, NY 10021

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Unlike imidazo[4,5-*d*]pyridazin-4(5*H*)-one (**1a**), which undergoes ribosylation at N-6 in the Vorbruggen procedure for nucleoside synthesis, the 5-benzyloxymethyl derivative **12** undergoes ribosylation at N-1 and N-3 to give a separable mixture of **14** and **15**. Removal of the N-5 blocking groups from **14** and **15** by treatment with boron trichloride at -78° affords the intermediates **16** and **17**, which were debenzoylated to give the 4-oxo nucleosides **5** and **6**. Thiation of **16** and **17**, followed by *S*-methylation and ammonolysis leads to the 4-amino nucleosides **2** and **3**. The glycosylation sites of these nucleosides were assigned by using a combination of ^{1}H and ^{13}C nmr data, especially measurements of the spin-lattice relaxation times (T_1) of the base protons. Using these techniques, it is shown that a nucleoside previously reported to be **3** is in fact the N-6 isomer.

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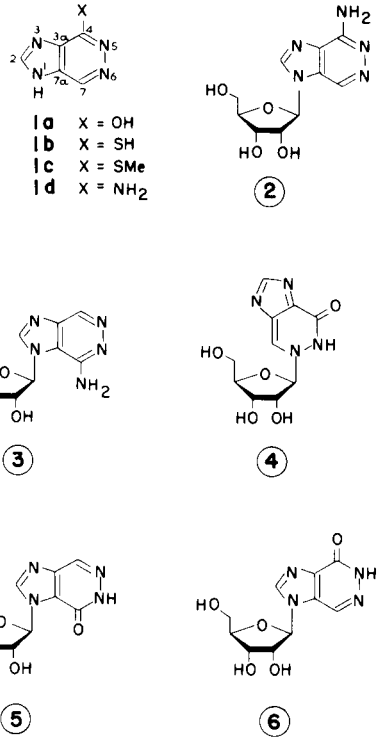
The search for antimetabolites structurally related to the naturally-occuring purines has prompted the synthesis of numerous imidazo[4,5-*d*]pyridazines [1], a ring-system that results from the interchange of the purine N-3 and C-2 positions. Some 4-substituted imidazo[4,5-*d*]pyridazines do, in fact, function as substrates and inhibitors of purine-requiring enzymes. For example, the adenine analog **1d** [2], scheme 1, competitively inhibits adenine deaminase from yeast [3], and is a substrate for the adenine phosphoribosyl transferases from human erythrocytes [4] and Ehrlich ascites cells [5]. In addition, the analog (**1d**) is cytotoxic to mouse leukemia cells (L1210, L5178Y) and human epidermoid cells (H.Ep #2) *in vitro* [4,6].

Scheme I

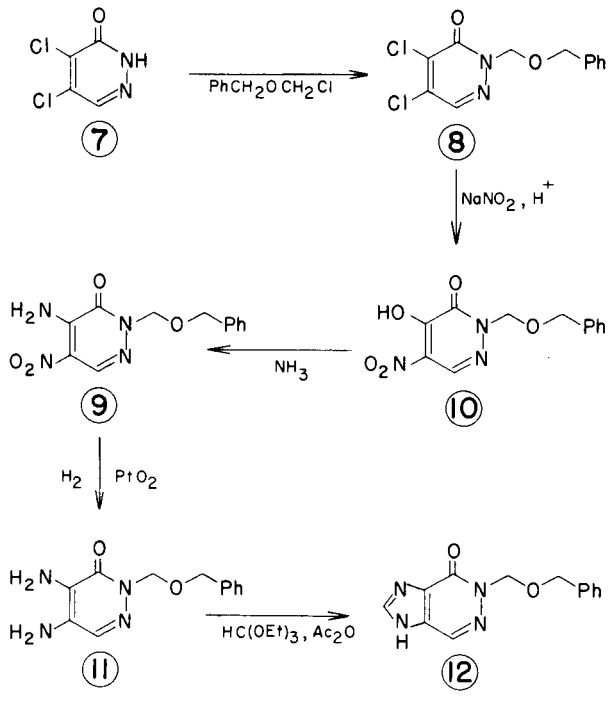


In spite of these biological activities, imidazo[4,5-*d*]pyridazine nucleosides have received only limited attention. In the area of 4-amino nucleosides, Carbon [7] has described the synthesis of compounds assigned the N-1 (**2**) and N-3 (**3**) structures. However, these structures require verification because of uncertainties in the method used to assign the glycosylation sites. A re-examination of the data, made with the benefit of hindsight, suggests that the N-3 structure is not definitely excluded for **2**, and that **3** is more likely ribosylated on one of the pyridazine nitrogens [8]. In the area of 4-oxo nucleosides, Cook and coworkers [9] have prepared the N-6 isomer **4** *via* a condensation reaction and the N-3 isomer **5** *via* a multistep procedure. We have recently given a preliminary account [10] that describes an alternative route to **5** and the first synthesis of the inosine analog **6**.

In order to clarify the structures of the 4-aminoimidazo-[4,5-*d*]pyridazine nucleosides we have now converted the 4-oxo nucleosides **5** and **6** into their 4-amino counterparts. These conversions, together with details of the synthesis of the oxo nucleosides (**5** and **6**) and nmr proofs of structure, form the subject of the present paper.

Since Cook and coworkers [9] had already shown that condensation of the bis-TMS derivative of **1a** with 1-*O*-acetyl-tri-*O*-benzoylribofuranose leads to the N-6 isomer **4**, rather than the desired N-1 isomer **6**, we reasoned that incorporation of a large but removable blocking group at N-5 of the base might influence the site of glycosylation [11]. Fortunately, the standard synthesis [12] of imidazo[4,-5-*d*]pyridazine-4(5*H*)-ones from pyridazines allows for the convenient introduction of 5-substituents, and we have therefore used this route (Scheme 2) to prepare the 5-benzyloxymethyl derivative **12**. Our working hypothesis was

Scheme 2



that if **12** silylates on oxygen to give **13**, then the sites of glycosylation might be limited by mechanistic and steric considerations to N-1 and N-3. This is apparently the case, because condensation of silylated **12** (Scheme 3) with 1-*O*-acetyl-tri-*O*-benzoylribofuranose in the presence of stannic chloride affords two products, **14** and **15**, which proved to be the N-1 and N-3 nucleosides, respectively. The relative amounts of **14** and **15** formed in this reaction depend on the amount of stannic chloride used and on the time-scale of the reaction. With small amounts of catalyst, it was possible to show by hplc analysis that the first-formed **15** is converted partially into **14** when the reaction mixture is stored at room temperature. Similar isomerizations have been observed by Vorbrüggen and coworkers [13]. Under optimum conditions, **14** and **15** were obtained from **12** in yields of 48 and 40%, respectively, after separation by preparative hplc. That both **14** and **15** are β-nucleosides was demonstrated by a debenzoylation-isopropylidenation sequence to afford products that satisfy Imbach's $\Delta\,\delta$ criterion for the β-configuration [14a], namely a 0.23 ppm separation of the isopropylidene methyl resonances in the pmr spectra. The appearance of the H-4′ resonances of these isopropylidene compounds as multiplets, rather than pseudotriplets, is also consistent [14b] with the β-configuration.

Attempts to remove the N-5 benzyloxymethyl groups from **14** and **15** by using ordinary catalytic hydrogenolysis gave highly erratic results. Transfer hydrogenolysis using cyclohexene as the hydrogen source in the presence of palladium-on-carbon was more successful, but the reactions are very slow and not suitable for routine use. However, treatment of **14** and **15** with boron trichloride in dichloromethane at -78° results in efficient cleavage of the benzyloxymethyl groups to give **16** and **17**. These reactions occasionally give some of the products where N-5 retains an hydroxymethyl substituent, but complete N-5 deblocking can be achieved by dissolving such intermediates in hot, aqueous pyridine. The fully deblocked nucleosides **5** and **6** were obtained by treating **17** and **16**, respectively, with ethanolic sodium hydroxide.

The 4-position of imidazo[4,5-*d*]pyridazine-4-ones appears to be less reactive than analogous positions in oxo purines and pyrimidines towards nucleophilic substitu-

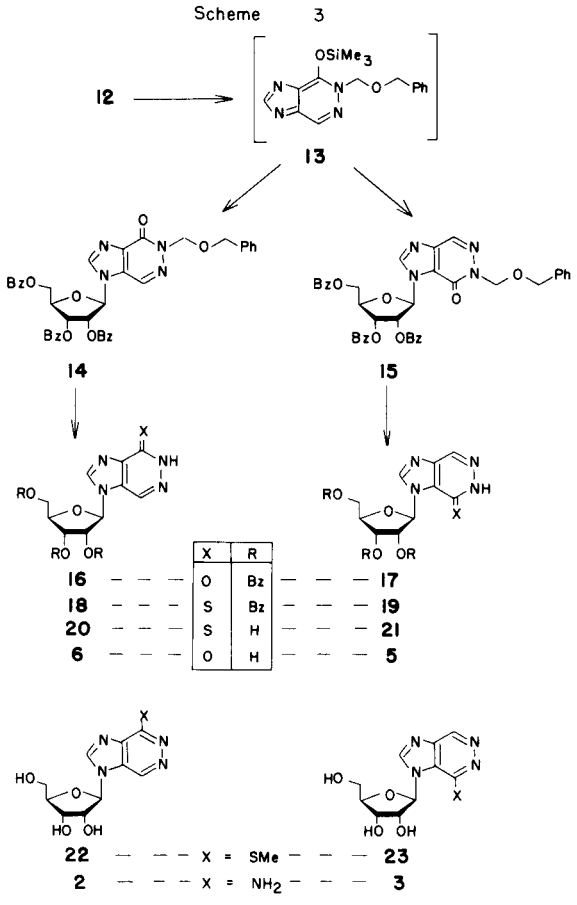
Table 1

^{13}C-NMR Data for Certain Imidazo[4,5-*d*]pyridazines

Compound	4	2	7	7a	3a	1′	2′	3′	4′	5′	$J_{C-7,H-7}$	$J_{C-2,H-2}$	$J_{C-2,H-1'}$
6	157.9	142.4	127.3	131.5	136.5	89.4	75.3	70.1	86.4	61.1	216	187	5
1a	156.3	143.4	131.8	138.9	129.5	—	—	—	—	—	211	187	—
5	155.2	143.9	133.7	142.1	125.6	89.2	75.5	69.8	85.6	60.9	215	188	5
20	175.9	144.5	131.6	126.4	146.0	89.6	75.3	69.9	86.5	60.9	217	194	4
1b	170.1	146.2	137.1	134.4	137.8	—	—	—	—	—	212	188	—
21	169.4	146.1	137.9	137.0	134.3	89.1	76.0	68.8	84.5	60.1	216	190	4
2	154.5	142.8	131.8	129.3	130.5	89.1	74.4	70.1	86.1	61.1	214	190	3
1d	151.7	149.5	135.3	138.3	129.0	—	—	—	—	—	202	182	—
3	149.5	144.3	138.6	142.2	120.1	89.2	75.3	69.3	86.4	60.5	213	184	4
24	152.6	159.4	131.1	141.1	136.8	100.6	75.4	68.7	85.4	60.1	197	187	—

Spectra were determined at 22° in methylsulfoxide-d_6 containing ~5% deuterium oxide, which was added to remove couplings involving exchangeable protons, for example J_{C-7,N^5-H} in the 4-oxo and thioxo compounds. Chemical shifts were measured relative to the central solvent peak, and then corrected to the TMS scale after determination of the methylsulfoxide-d_6 chemical shift under the operating conditions. The spectral width (4 KHz) and data point (8K) combination used gave a digital resolution of 0.97 Hz: the values of the three-bond couplings between C-2 and H-1′ are therefore approximate. The C-7a and C-3a assignments are based on the magnitude and direction of the shifts that are expected to result from replacement of a 4-oxo or amino substituent with thioxo.

Scheme 3



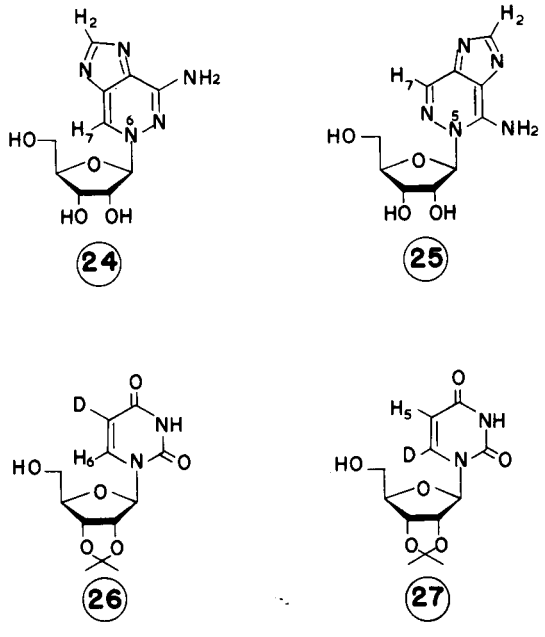
tion. Thus, the oxo nucleosides **16** and **17** failed to afford the 4-chloro derivatives when treated with the Vilsmeier reagent derived from thionyl chloride and dimethylformamide. Similarly, attempts to prepare the 4-triazole derivatives (a potential route to the 4-amines by ammonolysis) by treatment of **16** and **17** with 1,2,4-triazole and *p*-chlorophenylphosphodichloridate were not successful, although similar reactions proceed smoothly with *O*-esterified derivatives of deoxyguanosine and uridine [15]. However, **16** and **17** proved to be amenable to thiation, and treatment with phosphorus pentasulfide in hot pyridine leads to the 4-thiones **18** and **19** in yields of 60%. The use of phosphorus pentasulfide in dioxane or Lawesson's reagent [16], either of which normally thiate cyclic amides efficiently, proved to be unsatisfactory with **16** and **17**.

Methylation of the thiones **18** and **19** with an equivalent amount of methyl iodide under alkaline conditions affords the methylthio nucleosides **22** and **23** in high yield, and subsequent ammonolysis of these materials leads to the 4-amines **2** and **3** in moderate yields. These ammonolyses require fairly drastic conditions (liquid ammonia, 150°, 4 hours) and are unusual in that glycosyl cleavage to afford some of the free base **1d** was observed in each case.

Determination of Glycosylation Sites.

In principle, *N*-glycosylation of the starting base **12** can lead to three nucleosides (N-1, N-3 and N-6), two of which (N-3, N-6) are known from the work of Cook *et al* [9]. By comparing the physical properties of our **5** and **6** with the literature values it was clear that **5** corresponds to the N-3 isomer but that **6** differs from the N-6 isomer **4**. Nucleoside **6**, by elimination, is therefore ribosylated at N-1, The 4-amino nucleoside **2** obtained *via* the route that also leads to **6** proved to be identical with a sample of **2** prepared by Carbon [17], indicating that his N-1 formulation is correct. As suspected, our N-3 amino nucleoside **3** is quite different from the material reported by Carbon to be the N-3 isomer [17]. Carbon's second isomer must therefore be ribosylated at either N-6 (**24**, Scheme 4) or N-5 (**25**).

Scheme 4



The foregoing structural assignments depend ultimately on comparisons with the N-3 and N-6 isomers of Cook *et al* [9], which were assigned by the ^{13}C-nmr α,β shift method. Although this method [18] is a powerful tool for assigning glycosylation sites, it sometimes gives misleading or anomalous results [19], and the authors of a recent review [19b] have commented that a certain amount of caution should be used in relying on this technique for unequivocal structure proofs. We have therefore used a variety of other nmr methods in order to establish structures without reference to previous assignments. The results offer independent evidence that the **2,6** and **3,5** assignments are indeed correct, and indicate that Carbon's second isomer is compound **24**.

Table 2

Proton Chemical Shifts and Coupling Constants of Certain Imidazo[4,5-*d*]pyridazines

Compound	2	7	1′	2′	3′	4′	5′	OH	OH	OH	Other
6	8.52	8.66	5.90 d (5.8)	——4.0-4.3 m——			3.67 m	5.57 d (5.1)	5.30 d (4.0)	5.23 t (5.2)	NH, 12.74 bs
1a	8.39	8.41	—	—	—	—	—	—	—	—	NH, 12.75 bs
5	8.78	8.43	6.38 d (5.3)	4.39 m	4.14 m	3.96 m	3.64 m	5.50 d (5.8)	5.19 d (4.9)	5.17 t (4.9)	NH 12.90 bs
20	8.69	9.05	5.95 d (6.1)	——4.0-4.3 m——			3.67 m	5.60 d (5.7)	—5.31 m—		NH, 14.31 bs
1b	8.54	8.82	—	—	—	—	—	—	—	—	NH, 14.37 bs
21	9.08	8.83	7.46 d (4.0)	4.31 m	4.17 m	3.96 m	3.68 m	5.44 d (5.5)	5.22 d (4.9)	5.14 t (5.5)	NH, 14.40 bs
22	8.77	9.67	6.01 d (6.1)	4.31 m	—4.0-4.2 m—		3.68 m	5.75 d (5.8)	—5.33 m—		SMe, 2.73
1c	8.56	9.37	—	—	—	—	—	—	—	—	SMe, 2.75
23	9.02	9.42	6.35 d (4.9)	4.46 m	4.19 m	4.02 m	3.66 m		——5.5 m——		SMe, 2.80
2	8.55	9.11	5.90 d (6.4)	4.31 m	4.08 m		3.70 m	5.57 d (6.1)	5.30 d (4.3)	5.22 t (5.2)	NH_2, 6.59 bs
1d	8.24	8.78	—	—	—	—	—	—	—	—	NH_2, 6.98 bs
3	8.65	8.94	5.99 d (6.1)	——4.0-4.3 m——			3.67 m		——5.29 m——		NH_2, 6.35 bs
24	8.23	9.61	5.81 d (2.1)	——4.1-4.3 m——			3.75 m	5.73 d (4.9)	5.22 d (5.5)	5.28 t (5.5)	NH_2, 6.99 bs

Coupling constants (hz) are given in parenthesis. Peaks are sharp singlets unless designated bs (broad singlet), d (doublet), t (triplet) or m (multiplet). For the free bases, the imidazole N-1(N-3) proton was apparent only for the 4-thioxo compound **1b** (δ 14.01 bs). For compounds also studied by ^{13}C nmr, selective decoupling was used to assign H-7 and H-2, except for **6**, **1a** and **21** where the H-resonances are too close together. H-7 in **21** was readily assigned on the basis of its large T_1 value: for the other compounds, the base proton peaks were assigned in the sequence H-7—H-2. Qualitative T_1 measurements (data not shown) confirmed this sequence for **23**.

Table 3

T_1 Values (seconds) of the Base Protons of Certain Imidazo[4,5-*d*]pyridazines

Compound	Concentration	H-2	H-7
1a	25 m*M*	10.1	13.1
5	"	0.9	9.0
6	"	1.0	0.8
2	5 m*M*	1.2	1.3
3	"	1.1	14.4
24	"	6.1	0.7
		H-5	H-6
26	25 m*M*	—	0.9
27	"	~10	—

T_1 values were measured at 22° by the inversion-recovery method in methylsulfoxide-d_6 containing (except for **5** and **6**) ~5% deuterium oxide. Solutions were degassed by five freeze-pump-thaw cycles before the tubes were sealed. The pulse sequences were separated by delay times at least five fold that of the longest T_1, except for **27**, where the interval was 2.5 fold.

Table 4

Downfield Shifts (ppm) of Nucleoside H-2 and H-7 Resonances Relative to the Free Bases

N-1 isomers			N-3 isomers		
Compound	H-7	H-2	Compound	H-7	H-2
2	0.33	0.31	**3**	0.16	0.41
6	0.25	0.13	**5**	0.02	0.39
20	0.23	0.15	**21**	0.01	0.54
22	0.30	0.21	**23**	0.05	0.46
mean	0.28	0.20	mean	0.06	0.45

NMR Peak Assignments.

The following nmr methods require the unambiguous assignments of the H-2,C-2 and H-7,C-7 resonances of the various imidazo[4,5-*d*]pyridazine nucleosides. Although reliable assignments of H-2 and H-7 cannot be made simply by inspection, the C-2 and C-7 resonances can be identified without difficulty. In each case, C-2, which bears an extra nitrogen atom, resonates downfield of C-7 (Table 1), and this sequence is confirmed by the size of the one-bond coupling constants. Thus the splitting of the C-2 signals (average ^1J = 211 Hz) is larger than that of the signals assigned to C-7 (average ^1J = 188 Hz), and these coupling constants are in excellent agreement with the values predicted by Malinowski's additivity rules [20], namely $J_{C\text{-}7,H\text{-}7}$ = 187.5 and $J_{C\text{-}2,H\text{-}2}$ = 211 Hz. With the sequence firmly established as C-2 — C-7 (from downfield to upfield), selective decoupling experiments were used to determine the chemical shifts of H-2 and H-7, except where noted in table 2. The base-proton resonance sequence for most of the imidazo[4,5-*d*]pyridazines listed in Table 2 is H-7 — H-2, which agrees with previous assignments of the parent ring system [12a]. However, this sequence is reversed for the nucleosides **5** and **21**, and their precursors, and we will return to this point in a later section.

Long-range ^{13}C- ^1H Coupling.

Analysis of the three-bond couplings between H-1′ and the carbons α to the glycosylated nitrogen can lead to unequivocal nucleoside structural assignments [18c]. In our case, it was clear from the coupled spectra that the nucleo-

sides derived from **14** and **15** are ribosylated on an imidazole nitrogen because of small couplings (3-5 Hz) between C-2 and H-1′. However, we have not been able to differentiate between N-1 and N-3 substitution on the basis of couplings between H-1′ and C-3a or C-7a, primarily because the weak signals of these bridgehead carbons, and frequent overlap with the much more intense C-2 and C-7 multiplets, preclude the measurement of reliable coupling constants. Unfortunately, coupling between C-7 and H-1′ or between C-4 and H-1′, which would distinguish between the N-6 (**24**) and N-5 (**25**) isomers, was not observed for Carbon's second isomer [17] under the conditions used. These three-bond couplings apparently depend on the glycosyl torsion angle [21], so it is possible that the lack of observable coupling in this case simply reflects a situation where the rotation of the base corresponds to a region of minimum coupling.

Proton Spin-Lattice Relaxation.

The most useful nmr technique for differentiating between isomeric imidazo[4,5-*d*]pyridazine nucleosides has proved to be the measurement of the spin-lattice relaxation times, T_1, of the base protons. For compounds in low concentration in magnetically inert solvents, the mechanism of proton spin-lattice relaxation is dominated by dipole-dipole interactions between neighbouring protons in the same molecule [22]. The efficiency of these interactions, and hence the size of T_1, is highly dependent on the interproton distances. For nucleosides in general this means that protons on the base that can approach those of the sugar ring by glycosyl rotation are expected to have short T_1 relaxation times. Conversely, much larger T_1 values are expected for solitary base protons that are remote from the sugar protons - in this case, intermolecular processes become important in the relaxation mechanism. Applying these principles to the nucleosides listed in Table 3, it is seen that the ten-fold difference in the T_1 values of H-2 and H-7 in the 4-oxo nucleoside **5** indicates that H-2 is relaxed efficiently by interactions with the sugar ring protons, but that H-7 occupies a remote position, namely the N-3 structure. In contrast, the T_1 values of both H-2 and H-7 in isomer **6** are similar and small, indicating that both protons are close to the sugar ring. This situation can be achieved only in the N-1 structure, where H-7 can interact specifically with H-1′ in the *anti* conformation, or with other ribosyl protons via a *syn-anti* equilibrium. As expected, the absence of the sugar moiety, as in the free base **1a**, results in large T_1 values for both H-2 and H-7. With regard to the 4-amino nucleosides, the T_1 values found for **2** and **3** similarly indicate the N-1 and N-3 structures, respectively. For Carbon's second isomer, originally thought to be **3**, the T_1 values indicate that H-7 is close to and H-2 remote from the sugar ring, a situation that is accomodated only by the N-6 structure **24** [23]. If the compound was instead the N-5 isomer **25**, then the T_1 value of H-7 would be expected to substantially exceed the 0.7 seconds actually observed. This conclusion is supported by the T_1 values found for some model compounds, namely the partially deuterated isopropylideneuridines **26** and **27**. Thus H-5 of **27** [24], which can occupy a position with respect to the sugar ring similar to that of H-7 in **25**, shows a substantial T_1 value of ~ 10 seconds. On the other hand, H-6 in **26** [24] approximates the location of H-7 in **24**, and the T_1 values of 0.9 and 0.7 seconds, respectively, are accordingly similar [25].

Proton spin-lattice relaxation times have been used extensively to investigate conformational preferences, stacking interactions, H-bonding and anomeric configurations in a variety of nucleosides and nucleotides [26-29], but we are not aware of other examples of the use of this technique for determining glycosylation sites.

Proton Chemical Shifts.

The structure assignments derived from the T_1 measurements are also supported by chemical shift data for H-1′ and the base protons (Table 2). Considering first the anomeric position, a large body of data obtained with a variety of nucleosides [30] show that carbonyl and thiocarbonyl groups adjacent to H-1′ cause a substantial deshielding effect, relative to the case where these groups are widely separated. Accordingly, the 0.48 ppm downfield shift of H-1′ of **5** relative to **6** is consistent with the N-3 structure for **5** and the N-1 structure for **6**. Similar conclusions follow from the even larger deshielding (1.51 ppm) of H-1′ in **21** relative to **20**.

With regard to the chemical shifts of H-2 and H-7, previous studies [31] with purine nucleosides have suggested that the ribofuranose ring exerts a deshielding effect on base protons adjacent to the site of glycosylation, and a similar effect is evident from the data shown in Table 2. As summarized in Table 4, the H-2 resonances of the nucleosides derived from **15** (**3**, **5**, **21** and **23**) are deshielded relative to their positions in the free bases by an average of 0.45 ppm, whereas smaller differences (average 0.2 ppm) are noted for the nucleosides derived from **14** (**2**, **6**, **20** and **22**). These differential shieldings [32] are consistent with the assigned N-1 and N-3 structures because of conformational differences expected between the two series. For the N-3 isomers, the conformation is expected to be restricted to the *anti* range because of the presence of both the 4-substituent and the sugar ring on the same side of the molecule. H-2 is held directly above the sugar ring in this conformation, and therefore fully exposed to the deshielding effect. For the N-1 isomers, greater mobility about the glycosyl bond is expected to produce a different time-averaged conformation in which H-2 is free to move away from the area of the sugar ring. The data in Table 4

reveal similar differences for the chemical shifts of the H-7 protons. For the N-3 isomers **3, 5, 21** and **23**, the H-7 protons are hardly affected by the presence of the ribose moieties, moving downfield on average by only 0.06 ppm relative to the free bases. Conversely, the H-7 resonances of the N-1 isomers **2, 6, 20** and **22** are deshielded by an average of 0.28 ppm, a value that suggests a significant population of *syn* rotamers for these nucleosides. The differential shielding of H-2 and H-7 explain why the usual base-proton chemical shift sequence (δ H-7 > δ H-2) is reversed for the N-3 nucleosides **5** and **21**. Only in these particular nucleosides are the downfield shifts of H-2 minus those of H-7 greater than the distances separating the H-7 and H-2 resonances in the respective free bases (**1a** and **1b**).

It should also be noted that H-2 of **24** and **1d** have closely similar chemical shifts, whereas H-7 of **24** is substantially deshielded (0.82 ppm). Although some of this difference might result from the different tautomeric structure of **24**, the overall trend is consistent with the N-6 structure.

Now that the structures of the 4-aminoimidazo[4,5-*d*]-pyridazine nucleosides have been clarified, we can summarize the little that is known of their biological properties. The adenosine analog **2**, designated as 2-aza-3-deaza-adenosine, has been shown recently to be an excellent substrate but a mediocre inhibitor of *S*-adenosylhomocysteine hydrolase from beef liver [33]. The analog (**2**) is not appreciably cytotoxic towards H.Ep #2 cells [6b], implying that the compound is not a substrate for adenosine kinase. Surprisingly, the isomer previously thought to be **3**, but now shown to be **24**, is moderately cytotoxic towards H.Ep #2 cells, and it was suggested [6b] from studies with resistant cell lines that this activity is due to cleavage to the free base. This explanation probably stands, although whether the cleavage is enzyme-mediated or spontaneous remains to be seen. Studies on the biological activities of the new analogs are in progress.

EXPERIMENTAL

General Procedures.

Melting points were determined on a Thomas-Hoover apparatus (capillary method) and are uncorrected. Ultraviolet spectra were measured on Cary model 15 and Unicam SP-800 spectrometers. Thin layer chromatography (tlc) was performed on 250 μm silica gel GF_{254} plates (2.8 × 8 cm., Analtech, Inc.) and separated materials were detected with ultraviolet light and/or spraying with sulfuric acid in ethanol (10%, v/v) followed by charring. Flash chromatography [34] was performed on Merck Silica Gel 60 (230-400 mesh ASTM). Preparative hplc was performed on a Waters 500 Prep LC system using silica gel cartridges. Nmr spectra were obtained with a JEOL PFT-100 instrument operating at 100 MHz for ^1H and 25.15 MHz for ^{13}C spectra. Dry methylsulfoxide-d_6 was used as solvent for ^1H spectra, with tetramethylsilane as internal standard. Other operating conditions are noted in Tables 1-3. Microanalyses were performed by Spang Microanalytical Laboratory, Eagle Harbor, Michigan; Galbraith Laboratories, Knoxville, Tennessee; and M. H. W. Laboratories, Phoenix, Arizona. All evaporations were carried out *in vacuo*. Samples for analysis were dried *in vacuo* at room temperature, unless otherwise stated, and nmr confirmation was obtained for those compounds that are reported as solvates.

4,5-Dichloro-2-benzyloxymethylpyridazin-3-one (**8**).

A solution of 2.0 g (0.12 mole) of **7** (35) in 75 ml of dimethylformamide containing 2.3 ml (0.13 mole) of 1,8-diazabicyclo[5.4.0]undec-7-ene was cooled to 0°. Benzyl chloromethyl ether [36] (22.2 ml, 0.16 mole) was added in one portion, the ice bath was removed, and the reaction mixture was stirred for 2 hours. After removal of solvents, the residue was partitioned between water and dichloromethane (300 ml each) and the separated aqueous phase was extracted with two additional 150 ml portions of dichloromethane. The combined organic extracts were dried over sodium sulfate. The filtrate was concentrated to dryness and the brown residue was decolorized by flash chromatography, using dichloromethane as eluant. The appropriate fractions were combined and concentrated to give a colorless solid, which was recrystallized from ethanol to afford 19.95 g (58%) of **8** in two crops, mp 72-72.5°; nmr: δ 8.21 (s, 1H, H-6), 7.31 (s, 5H, Ph), 5.50 (s, 2H, N-CH_2-O) and 4.65 (s, 2H, CH_2Ph).

Anal. Calcd. for $C_{12}H_{10}Cl_2N_2O_2$: C, 50.55; H, 3.54. Found: C, 50.85; H, 3.69.

4-Hydroxy-5-nitro-2-benzyloxymethylpyridazin-3-one (**10**).

A solution of 12 g (0.17 mole) of sodium nitrite in 40 ml of water was added to a solution of **8** (12 g, 42 mmoles) in 150 ml of dimethylformamide, and the mixture was heated at 80-90° for 24 hours. After cooling, the reaction mixture was evaporated to dryness and the residue was dissolved in 50 ml of warm 6*N* hydrochloric acid. Pale yellow crystals of **10** (10.3 g, 93%) separated on cooling. A sample recrystallized from methanol for analysis was essentially colorless, mp 149-151°; nmr: δ 11.45 (bs, 1H, OH), 8.29 (s, 1H, H-6), 7.31 (s, 5H, Ph), 5.39 (s, 2H, N-CH_2-O) and 4.62 (s, 2H, CH_2Ph).

Anal. Calcd. for $C_{12}H_{11}N_3O_5$: C, 51.99; H, 3.99. Found: C, 51.93; H, 4.07.

4-Amino-5-nitro-2-benzyloxymethylpyridazin-3-one (**9**).

A solution of 9.0 g (0.032 mole) of **10** in 150 ml of methanolic ammonia (saturated at 0°) was heated at 130° in a sealed, glass-lined steel bomb for 22 hours. Bright yellow crystals of pure **9** (7 g, 80%) separated on cooling, mp 122-124°; nmr: δ 8.93 (bs, 2H, NH_2), 8.33 (s, 1H, H-6), 7.31 (s, 5H, Ph), 5.44 (s, 2H, N-CH_2-O), and 4.64 (s, 2H, CH_2Ph).

Anal. Calcd. for $C_{12}H_{12}N_4O_4$: C, 52.17; H, 4.38. Found: C, 52.17; H, 4.43.

4,5-Diamino-2-benzyloxymethylpyridazin-3-one (**11**).

A solution of 2.13 g (7.8 mmoles) of **10** in a mixture of ethanol (180 ml) and ethyl acetate (40 ml) was hydrogenated at 2 atmospheres of hydrogen in the presence of platinum oxide (~100 mg) for 30 minutes at room temperature. The mixture was filtered through a pad of celite and the filtrate was concentrated to afford colorless **11** (1.63 g, 85%), which was homogenous by tlc (chloroform-methanol, 9:1, v/v). Compound **11** crystallizes readily from absolute ethanol, mp 175-176°; nmr: δ 7.48 (s, 1H, H-6), 7.31 (s, 5H, Ph), 5.37 (s, 2H, N-CH_2-O), 5.30 (bs, 2H, NH_2), 5.01 (bs, 2H, NH_2), and 4.59 (s, 2H, CH_2Ph).

Anal. Calcd. for $C_{12}H_{14}N_4O_2$: C, 58.52; H, 5.73; N, 22.75. Found: C, 58.60; H, 5.96; N, 22.75.

5-Benzyloxymethylimidazo[4,5-*d*]pyridazin-4-one (**12**).

A mixture of 3.34 g (0.014 mole) of **11**, 47 ml of triethyl orthoformate and 47 ml af acetic anhydride was heated under reflux for 1 hour. After cooling, the reaction mixture was evaporated to dryness. Crystallization of the resulting residue from 95% ethanol afforded **12** in partially hydrated form, mp 194-195°; nmr: δ 8.48 (s, 1H, H-7), 8.41 (s, 1H, H-2), 7.30 (s, 5H, Ph), 5.59 (s, 2H, N-CH_2-O) and 4.65 (s, 2H, CH_2Ph).

Anal. Calcd. for $C_{13}H_{12}N_4O_2 \cdot 0.25\ H_2O$: C, 59.88; H, 4.83; N, 21.40. Found: C, 59.75; H, 4.90; N, 21.21.

With more prolonged reaction times, the product obtained was found by nmr to contain an *N*-acetyl group (δ 2.79). This material can be converted into **12** by treatment with methanolic ammonia at room tempera-

ture for 3 hours.

1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-5-benzyloxymethylimidazo[4,5-*d*]pyridazin-4-one (**14**) and 3-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-5-benzyloxymethylimidazo[4,5-*d*]pyridazin-4-one (**15**).

A sample of **12** (11.76 g, 0.46 mole) was suspended in hexamethyldisilazane (150 ml) containing ammonium sulfate (5 mg), and the mixture was heated under reflux until all the materials had dissolved. The cooled solution was evaporated to dryness and the resulting syrup was dissolved in 500 ml of dry 1,2-dichloroethane. Solid 1-*O*-acetyl-2,3,5-*O*-benzoyl-β-D-ribofuranose (24.7 g, 0.049 mole) and stannic chloride (10 ml, 0.085 mole) were added, and the solution was stirred at room temperature for 3 hours. The reaction mixture was then poured onto 1 l of saturated sodium bicarbonate solution and stirring was continued for an additional 30 minutes. After filtration through a pad of celite, the organic phase was separated, washed with water, and dried over sodium sulfate. Concentration afforded 37.4 g of crude product, which was fractionated by preparative hplc (ethyl acetate-*n*-hexane, 3:2, v/v) to afford 12.7 g (40%) of **15** and 15.3 g (48%) of **14**, both of which were sufficiently pure for further reactions. Analytically pure samples of **14** and **15** were obtained as colorless foams after flash chromatography (*N*-hexane-ethyl acetate, 2:1, v/v); nmr data for **14**: δ 8.74 and 8.71 (two s, 2H, H-7 and H-2), 8.03-7.44 (m, 15H, Bz), 7.27 (s, 5H, Ph), 6.78 (d, 1H, H-1', $J_{1',2'}$ = 4.4 Hz), 6.03 (m, 2H, H-3' and H-2'), 5.56 (s, 2H, N-CH_2-O), 4.85 (m, 3H, H-4' and H-5'ab), and 4.61 (s, 2H, CH_2Ph).

Anal. Calcd. for $C_{39}H_{32}N_4O_9$: C, 66.85; H, 4.60. Found: C, 67.00; H, 4.61.

The nmr data [37] for **15** were: δ 8.82 (s, 1H, H-2), 8.56 (s, 1H, H-7), 8.05-7.40 (m, 15H, Bz), 7.26 (s, 5H, Ph), 6.88 (d, 1H, H-1', $J_{1',2'}$ = 4.3 Hz), 6.13 (m, 2H, H-2' and H-3'), 5.57 (ABq, 2H, N-CH_2-O, J_{gem} = 10.4 Hz), 4.81 (m, 3H, H-4' and H-5'ab) and 4.62 (s, 2H, CH_2Ph).

Anal. Calcd. for $C_{39}H_{32}N_4O_9$: C, 66.85; H, 4.60. Found: C, 66.96; H, 4.47.

The conversion of **14** and **15** into the 2',3'-*O*-isopropylidene compounds required for nmr determination of their anomeric configurations was carried out as follows. The samples of **14** or **15** (100 mg) were debenzoylated by treatment with sodium (100 mg) in methanol (15 ml) for 1 hour, sodium ions were removed with excess Dowex 50(H+), and the filtrate and washings were concentrated to dryness. The residue was suspended in acetone (2 ml) containing dimethoxypropane (2 ml) and *p*-toluenesulfonic acid (5 mg), and the mixture was stirred vigorously for 1.5 hours. After neutralization with solid sodium bicarbonate, the mixture was filtered, reduced in volume and the isopropylidenated product was isolated by preparative tlc (500 μm, 20 × 20 cm plate) using chloroform-methanol, 15:1, v/v as developing agent. The nmr data for 1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-5-benzyloxymethylimidazo[4,5-*d*]pyridazin-4-one obtained from **14** were: δ 8.63 (s, 1H, H-7), 8.60 (s, 1H, H-2), 7.31 (s, 5H, Ph), 6.26 (d, 1H, H-1', $J_{1',2'}$ = 3.1 Hz), 5.60 (s, 2H, N-CH_2-O), 5.22 (dd, 1H, H-2', $J_{2',3'}$ = 6.1 Hz), 4.97 (dd, 1H, H-3', $J_{3',4'}$ = 2.4 Hz), 4.64 (s, 2H, CH_2Ph), 4.28 (m, 1H, H-4'), 3.50 (pseudo d, 2H, H-5'ab), 1.57 and 1.35 (two 3H s, isopropylidene methyls Δ δ 22.6 Hz). The nmr data for 3-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-5-benzyloxymethylimidazo-[4,5-*d*]pyridazin-4-one obtained from **15** were: δ 8.76 (s, 1H, H-2), 8.52 (s, 1H, H-7), 7.30 (s, 5H, Ph), 6.60 (d, 1H, H-1', $J_{1',2'}$ = 2.9 Hz), 5.56 (s, 2H, N-CH_2-O), 5.20 (dd, 1H, H-2', $J_{2',3'}$ = 6.3 Hz), 4.95 (dd, 1H, H-3', $J_{3',4'}$ = 2.9 Hz), 4.66 (s, 2H, CH_2Ph), 4.23 (m, 1H, H-4'), 3.59 (pseudo d, 2H, H-5'ab), 1.55 and 1.33 (two 3H s, isopropylidene methyls, Δ δ, 22.6 Hz).

1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)imidazo[4,5-*d*]pyridazin-4-(5*H*)-one (**16**).

A solution of 719 mg (1.03 mmoles) of **14** in 36 ml of dichloromethane was cooled to -78°. A pre-cooled (-78°) solution of boron trichloride in dichloromethane (7.8 ml of 1*M* solution) was added in one portion, and the mixture was stirred at -78° for 2 hours. Boron trichloride was hydrolyzed by the addition of methanol in dichloromethane (40 ml of 1:1, v/v mixture) with stirring, first at -78° for 2 hours and then at room temperature for an additional 30 minutes. The reaction mixture was concentrated to dryness and methanol was added to and evaporated from the residue to ensure the removal of boron-containing hydrolysis products as methyl borate. Flash chromatography (chloroform-methanol, 20:1, v/v) of the residue afforded 494 mg (79%) of **16**, which crystallized from methanol, mp 222-224°; nmr: δ 12.81 (bs, 1H, NH), 8.69 (s, 1H, H-7), 8.64 (s, 1H, H-2), 8.0-7.4 (m, 15H, Bz), 6.75 (1H, broadened by virtual coupling, H-1', $J_{1',2'}$ ~ 4.6 Hz), 6.00 (m, 2H, H-2' and H-3') and 4.82 (m, 3H, H-4', H-5'ab).

Anal. Calcd. for $C_{31}H_{24}N_4O_8$: C, 64.13; H, 4.17. Found: C, 63.88; H, 4.20.

3-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)imidazo[4,5-*d*]pyridazin-4-(5*H*)-one (**17**).

Treatment of **15** (1 g, 1.43 mmoles) with boron trichloride (11 ml of 1*M* solution in dichloromethane), and isolation of the product as described above for **16**, afforded 445 mg (51%) of **17**, which crystallized from methanol, mp 182-183°; nmr: δ 13.05 (bs, 1H, NH), 8.78 (s, 1H, H-2), 8.48 (s, 1H, H-7), 8.05-7.40 (m, 15H, Bz), 6.84 (d, 1H, H-1', $J_{1',2'}$ = 4.9 Hz), 6.10 (m, 2H, H-2' and H-3'), 4.92-4.68 (m, 3H, H-4' and H-5'ab).

Anal. Calcd. for $C_{31}H_{24}N_4O_8$: C, 64.13; H, 4.17. Found: C, 64.13, H, 4.06.

1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)imidazo[4,5-*d*]pyridazin-4(5*H*)-thione (**18**).

Phosphorus pentasulfide (14 g) was added to a solution of **16** (9.31 g, 0.015 mole) in 250 ml of pyridine containing 2 ml of water, and the mixture was heated under reflux for 12 hours. After cooling, the supernatant was decanted and the residue was washed with three 100 ml portions of pyridine. The supernatant and washings were evaporated to an oil which was partitioned between chloroform and 10% aqueous sodium bicarbonate solution. The chloroform layer was washed, dried (sodium sulfate), and evaporated to give a foam. Flash chromatography (hexane-ethyl acetate, 1:1, v/v) afforded 5.43 g (60%) of **18**, which was homogeneous on tlc (chloroform-methanol, 30:1, v/v). Colorless needles of **18** were obtained from methanol, mp 112-115°; nmr: δ 14.37 (bs, 1H, NH), 9.01 (s, 1H, H-7), 8.84 (s, 1H, H-2), 8.03-7.37 (m, 15H, Bz), 6.78 (d, 1H, H-1', $J_{1',2'}$ = 4.6 Hz), 6.03 (m, 2H, H-2' and H-3'), and 4.84 (m, 3H, H-4' and H-5'ab).

Anal. Calcd. for $C_{31}H_{24}N_4O_7S$; C, 62.41; H, 4.06. Found: C, 62.29; H, 4.39.

3-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)imidazo[4,5-*d*]pyridazin-4(5*H*)-thione (**19**).

Thiation of **17** as described above for **16** afforded **19** as colorless crystals (from ethanol) in 60% yield, mp 214-215°; nmr: δ 14.49 (bs, 1H, NH), 9.08 (s, 1H, H-2), 8.89 (s, 1H, H-7), 8.25 (d, 1H, H-1', $J_{1',2'}$ = 4.3 Hz), 8.07-7.44 (m, 15H, Bz), 6.12 (m, 2H, H-2' and H-3') and 4.84 (m, 3H, H-4' and H-5'ab).

Anal. Calcd. for $C_{31}H_{24}N_4O_7S$: C, 62.41; H, 4.06. Found: C, 62.07; H, 4.04.

1-(β-D-Ribofuranosyl)imidazo[4,5-*d*]pyridazine-4(5*H*)-one (**6**).

Sodium hydroxide solution (1.38 ml of 1*N*) was added to a solution of 200 mg (0.34 mmole) of **16** in 11 ml of 90% aqueous ethanol, and the mixture was stirred at room temperature for 2 hours. The solution was acidified to *p*H ~6 by the addition of glacial acetic acid and then concentrated to dryness. Fractionation of the residue by flash chromatography (chloroform-methanol, 5:1, v/v) and crystallization of the product from methanol afforded **6** (74.7 mg, 81%) as the hemi-methanolate, mp 200-202°, uv (*p*H 7): λ max 212 nm (ε 17300), 245 (ε 4840), 253 (ε 4800), 269 (ε 4700), λ min 234 (ε 4300), 248 (ε 4600), 257 (ε 4200); uv (*p*H 12): λ max 253 nm(ε 4900), 282 (ε 5300).

Anal. Calcd. for $C_{10}H_{12}N_4O_5 \cdot 0.5\ CH_3OH$: C, 44.36; H, 4.96; N, 19.71. Found: C, 44.40; H, 4.61; N, 19.35.

3-(β-D-Ribofuranosyl)imidazo[4,5-*d*]pyridazin-4(5*H*)-one (**5**).

Debenzoylation of **17** as described above for the N-1 isomer afforded 44.2 mg (49%) of **5**, mp ~120° dec, mp lit [9] dec >120°; uv (*p*H 7): λ max 212 nm (ε 13,250), 259 (ε 4200); λ min 229 (ε 1700); uv (*p*H 12): λ max 263 (ε 4700), λ min 233 (ε 2100). The ^{13}C and ^{1}H nmr data obtained for **5** are in good agreement with the literature [9] values.

1-(β-D-Ribofuranosyl)imidazo[4,5-*d*]pyridazin-4(5*H*)-thione (**20**).

Debenzoylation of **18** (195 mg, 0.33 mmole) was carried out as described for the 4-oxo analog **16**, except that the product crystallized on acidification of the reaction mixture. Recrystallization from 95% ethanol afforded 71 mg (84%) of **20**, mp 223-225°; uv (water): λ max 308 nm, λ min 260; uv (*p*H 12): λ max 295 nm, λ min 260.

Anal. Calcd. for $C_{10}H_{12}N_4O_4S \cdot 0.25\ H_2O$: C, 41.59; H, 4.36. Found: C, 41.71; H, 4.17.

3-(β-D-Ribofuranosyl)imidazo[4,5-*d*]pyridazin-4(5*H*)-thione (**21**).

Debenzoylation of **19** as described above for **18** afforded **21** in 90% yield, mp 224-226°; uv (water): λ max 323 nm (broad peak); λ min 265; uv (*p*H 12): λ max 304 nm, λ min 260.

Anal. Calcd. for $C_{10}H_{12}N_4O_4S$: C, 42.45; H, 4.26. Found: C, 42.11; H. 4.27.

1-(β-D-Ribofuranosyl)-4-methylthioimidazo[4,5-*d*]pyridazine (**22**).

A mixture of 7.54 g (12.6 mmoles) of **18**, 150 ml of 95% aqueous ethanol and 37.8 ml (37.8 mmoles) of 1*N* sodium hydroxide solution was stirred at room temperature for 2 hours. An additional equivalent of sodium hydroxide (12.6 ml of 1*N*) was then added and stirring was continued during the addition of 50.5 ml (12.6 mmoles) of a 0.25*M* solution of methyl iodide in ethanol [38]. After 1 hour, the *p*H of the reaction mixture was adjusted to ~6 with glacial acetic acid, and the solution was evaporated to dryness. Crystallization of the residue from ethanol afforded 3.55 g (94%) of **22**, mp 234-235°; uv (*p*H 1): λ max 236, 277, and 308 nm (pronounced shoulder), λ min 223 and 262 nm; uv (*p*H 7): λ max 227 and 273 nm, λ min 248 nm.

Anal. Calcd. for $C_{11}H_{14}N_4O_4S$: C, 44.29; H, 4.73. Found: C, 44.10; H, 4.89.

3-(β-D-Ribofuranosyl)-4-methylthioimidazo[4,5-*d*]pyridazine (**23**).

Debenzoylation of **19** and methylation of the intermediate **21** was carried out as described above for the synthesis of **20**. Flash chromatography of the crude product (chloroform-methanol; 5:1, v/v) and crystallization from methanol afforded **23** (86% yield) as the methanolate, mp 192-193°; uv (*p*H 1): λ max 240, 287, and 300 nm (shoulder), λ min 228 and 262 nm; uv (*p*H 7): λ max 224 and 280 nm, λ min 212 and 247 nm.

Anal. Calcd. for $C_{11}H_{14}N_4O_4S \cdot CH_3OH$: C, 43.63; H, 5.49. Found: C, 43.80; H, 5.43.

3-(β-D-Ribofuranosyl)-4-aminoimidazo[4,5-*d*]pyridazine (**3**).

Ammonia (~8 ml) was condensed in a tube containing 52.3 mg (0.18 mmole) of **23**, and the solution was heated in a steel bomb at 150° for 4 hours. After thorough cooling, the bomb was opened and vented in a hood. Flash chromatography (chloroform-methanol, 9:1, v/v) of the residue afforded, in order of elution, 9.4 mg (18%) of starting material **23**, 8.3 mg (35%) of the 4-amino free base **1d**, and 19.6 mg (42%) of **3**. Recrystallization of **3** from aqueous methanol afforded an analytical sample that retained water of crystallization even after heating at 100° *in vacuo* for 12 hours, mp 202-204°; uv (*p*H 7): λ max 214 nm (ε 20,350), 263 (ε 4900), λ min 234 (ε 2050); uv (*p*H 1): λ max 213 nm (ε 19,600), 258 (broad peak, ε 5900 shoulder at ~268). Longer reaction times result in increased formation of **1d** at the expense of **3**.

Anal. Calcd. for $C_{10}H_{13}N_5O_4 \cdot 1.5\ H_2O$: C, 40.81; H, 5.48. Found: C, 40.76; H, 5.18.

1-(β-D-Ribofuranosyl)-4-aminoimidazo[4,5-*d*]pyridazine (**2**).

Ammonolysis of **22** as described above for **23** afforded **2** in 30% yield, mp 225-226°, lit [7] 229-230°; uv (*p*H 7): λ max 213 nm (ε 19,100), 255 (ε 6500), 283 (shoulder, ε 4100), λ min 235 (ε 3700); uv (*p*H 1): 217 nm (ε 23,000), 252 (ε 8350), 257 (ε 8400), 277 (shoulder, ε 4200), min 240 (ε 7100). The uv data, as well as the ^{1}H and ^{13}C nmr spectra, are identical with those found for a sample [17] of **2** prepared by Carbon [7]. The free base **1d** is also formed in the ammonolysis of **22**.

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