IV. Reactions of *o*-, *m*-, and *p*-Lithiotoluenes with Excess *n*-BuLi–TMEDA. Solutions of *o*-, *m*-, and *p*-lithiotoluene were prepared by reacting 2.7 g (0.016 mol) of the appropriate bromotoluene with 0.3 g (0.04 g-atom) of lithium in 10 ml of dry ether. The solution of the lithiotoluene was transferred to 0.12 ml (8 mmol) of TMEDA in 10 ml of dry hexane. The ether was removed by distillation and 20 ml (0.032 mol) of 1.6 *M* *n*-BuLi in hexane was added. The resulting solution was refluxed for 16 hr. The dark colored solution and precipitate were quenched with 6.5 g (0.06 mol) of trimethylchlorosilane. The product mixture were worked up in the manner previously described.

A. ***p*-Lithiotoluene.** Glpc analysis of the residue showed **1p**, 82%; **2p**, 15%; and **3p**, 3%.

B. ***m*-Lithiotoluene.** Glpc analysis of the residue showed **1m**, 48%; **2m**, 28%; and **3m**, 24%.

C. ***o*-Lithiotoluene.** Glpc analysis of the residue showed **1o**, 90%; **2o**, 10%.

In a similar experiment, the *o*- and *p*-lithiotoluenes were allowed to react with excess *n*-BuLi–TMEDA at room temperature for 24 hr. No polysubstituted products could be detected by glpc after derivatization with trimethylchlorosilane.

V. Reaction of Bis(trimethylsilyl)phenylmethane (2a) with *n*-BuLi–TMEDA. Compound **2a**, 2.5 g (0.01 mol), was added to a mixture of 2.5 ml (0.02 mol) of 1.6 *M* solution of *n*-BuLi and 0.7 ml (5 mmol) of TMEDA. After 24 hr at room temperature, the yellow solution was quenched with 3.3 g (0.03 mol) of trimethylchlorosilane. The product mixture was worked up as described above to give 75% starting material **2a** and 23% compound **3o**. No other products were observable by glpc.

Acknowledgments. Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research.

Purine Nucleosides. XX. The Synthesis of 7-β-D-Ribofuranosylpurines from Imidazole Nucleoside Derivatives[1]

Robert J. Rousseau, Roland K. Robins, and Leroy B. Townsend

Contribution from the Department of Chemistry, University of Utah, Salt Lake City, Utah 84112. *Received December 18, 1967*

Abstract: A general route for the preparation of 7-glycosylpurines has been accomplished utilizing imidazole nucleosides. The utility of this synthetic route is demonstrated by the preparation of several 6- and 2,6-disubstituted 7-(β-D-ribofuranosyl)purines. The synthesis of the requisite imidazole nucleosides, ring closure procedures, assignment of anomeric configuration, and proof of the site of glycosidation of these β anomers is presented. A new and convenient preparation of 4-amino-5-carboxamido-1-(β-D-ribofuranosyl)imidazole (XV, iso-AICAR) has been accomplished *via* the fusion procedure. The synthesis of the β anomers of a number of 7-ribosylpurines, related to purine nucleosides isolated from naturally occurring vitamin B_{12} analogs which are obtained from certain microbiological sources, has now been achieved.

The first purine nucleoside isolated from a naturally occurring analog of vitamin B_{12} (pseudovitamin B_{12}) created considerable interest when it was characterized[2] as a 7-ribosylpurine.[3] This was the first example of a naturally occurring purine nucleoside which possessed the glycosyl moiety at any position other than N-9. A number of additional purines and purine ribosides were subsequently isolated from other naturally occurring analogs of vitamin B_{12} and characterized or presumed to be either 7-ribosylpurines or degradation products derived from 7-ribosylpurines.[4] This, of course, prompted active investigations in search of a general route for the synthetic preparation of 7-glycosylpurines. However, the laboratory synthesis of these compounds has been hindered by the inability[5] to develop a facile method for directing the carbohydrate moiety to N-7 rather than N-9 in the glycosidation of a preformed purine. The usual site of glycosidation in unsubstituted purines (no substituent on a ring nitrogen) has been shown[6] to occur primarily at N-9 with the only major exceptions being 7-glycosyltheophyllines[6,7] which possess a methyl group at N-1 and N-3. Other 7-glycosylpurines have recently been prepared[8,9] by using the directive influence,[10] probably steric, exerted by various 3-substituted purines. This approach possesses some inherent disadvantages,[2,10b] *viz.*, the initial preparation of appropriate 3-substituted purines and the numerous problems associated with the subsequent removal of the blocking groups at N-3. However, this latter approach has been the preferred method in view of the finding[11] that a number of reported 7-glycosylpurines were in actuality 3-glycosylpurines. This suggested that N-3 of a preformed purine should be blocked to ensure glycosidation at N-7. These difficulties were strong

(1) Supported by Contract No. PH-43-65-1041 with the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

(2) W. Friedrich and K. Bernhauer, *Chem. Ber.*, 89, 2507 (1956); W. Friedrich and K. Bernhauer, *Angew. Chem.*, 68, 580 (1956).

(3) It has been recently reported to possess the structure 6-amino-7-(α-D-ribofuranosyl)purine: J. A. Montgomery and H. J. Thomas, *J. Am. Chem. Soc.*, 85, 2672 (1963); J. A. Montgomery and H. J. Thomas, *ibid.*, 87, 5442 (1965).

(4) For a recent and comprehensive review of vitamin B_{12} and naturally occurring analogs of vitamin B_{12}, the reader is referred to R. Bonnett, *Chem. Rev.*, 63, 573 (1963); E. L. Smith, "Vitamin B_{12}," 2nd ed, Methuen Co., London, 1963; K. Bernhauer, O. Muller, and F. Wagner, *Angew. Chem. Intern. Ed. Engl.*, 3, 200 (1964).

(5) G. M. Blackburn and A. W. Johnson, *J. Chem. Soc.*, 4347 (1960).

(6) J. A. Montgomery and H. J. Thomas, *Advan. Carbohydrate Chem.*, 17, 301 (1962).

(7) W. A. Bowles and R. K. Robins, *J. Am. Chem. Soc.*, 86, 1252 (1964).

(8) J. A. Montgomery and H. J. Thomas, *J. Org. Chem.*, 28, 2304 (1963).

(9) H. J. Thomas and J. A. Montgomery, *ibid.*, 31, 1413 (1966).

(10) (a) A. D. Broom, L. B. Townsend, J. W. Jones, and R. K. Robins, *Biochemistry*, 3, 494 (1964); (b) J. A. Montgomery and H. J. Thomas, *J. Org. Chem.*, 30, 3235 (1965).

(11) L. B. Townsend, R. K. Robins, R. N. Loeppky, and N. J. Leonard, *J. Am. Chem. Soc.*, 86, 5320 (1964).

Table I. Ultraviolet Absorption of Certain Imidazole and Purine Nucleosides

No.	Compound	pH	λ_{max}, mμ	$\epsilon_{max} \times 10^{-3}$	λ_{min}, mμ	$\epsilon_{min} \times 10^{-3}$
I	5-Bromo-4-nitro-1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole	1	304	8.10	260	4.05
		MeOH	295	7.20	247	1.00
		11	232	9.00	262.5	2.23
			304	8.55		
	4-Bromo-5-nitro-1-(2′,3′,5′-tri-*O*-acetyl-D-ribofuranosyl)imidazole	1	312	6.75	270	3.15
		MeOH	309	6.75	270	3.15
		11	312	6.75	270	3.15
II	5-Cyano-4-nitro-1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole	1	292	6.75	258	3.57
		MeOH	286	6.33	249	3.16
		11	238	4.35	255	3.75
			292	6.35		
III	Methyl 4-nitro-1-(β-D-ribofuranosyl)imidazole-5-formimidate	1	289	3.62	255	2.56
		MeOH	289	5.72	255	2.56
		11	295	5.72	255	2.89
IV	5-Bromo-4-nitro-1-(β-D-ribofuranosyl)imidazole	1	307	7.13	260	2.26
		MeOH	299	6.80	260	2.26
		11	307	7.13	260	2.26
V	4-Amino-5-cyano-1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole	1	232	8.00		
			264	8.80	245	3.29
		EtOH	228	4.02	240	2.20
			269	10.60		
		11	228	4.02	239	3.66
			269	10.60		
XII	Methyl 4-nitro-1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-5-carboxylate	1	285	3.43	245	2.15
		MeOH	282	2.59	245	2.15
		11	...	...	...	...
XIII	5-Carboxamido-4-nitro-1-(β-D-ribofuranosyl)imidazole	1	296	5.47	253	2.30
		MeOH	292	5.77	248	2.74
		11	297	5.77	248	2.59
XV	5-Carboxamido-4-amino-1-(β-D-ribofuranosyl)imidazole	1	242	7.35	223	4.90
			268	7.49	253	6.58
		MeOH	238–248[a]	3.87	224	3.10
			279	9.55		
		11	272	13.40	226	5.81
IX	7-(β-D-Ribofuranosyl)adenine	1	220	11.50	240	6.41
			267	13.50		
		MeOH	213	15.70	234	4.00
			272	11.10		
		11	213	17.50	234.5	4.00
			271	9.35		
VII	2-Methyl-7-(β-D-ribofuranosyl)adenine	1	273	12.80	238	6.57
		MeOH	248	7.30	223	5.54
			276	8.50	257	6.13
		11	243	7.35	256	6.45
			275	8.98		
XVII	7-(β-D-Ribofuranosyl)hypoxanthine	1	250	9.00	228	3.62
		MeOH	258	9.37	235.5	4.29
		11	263	9.10	231	3.22
XIV	2-Methyl-7-(β-D-ribofuranosyl)hypoxanthine	1	253	9.90	228	4.20
		MeOH	260	8.50	231	3.00
		11	265	9.00	225	2.10
XVI	7-(β-D-Ribofuranosyl)hypoxanthine-2-thione	1	284.5	20.50	250.5	4.02
		MeOH	235	12.70	219	9.75
			282	18.10	252	5.10
		11	232	15.80	220	12.50
			273.5	17.40	251	8.80
XVIII	7-(β-D-Ribofuranosyl)guanine	1	251	8.44	230.5	5.41
			265–280[a]	6.63		
		MeOH	242.5	6.32	258	4.97
			283.0	6.62		
		11	227–240[a]	6.01	260.5	3.01
			281	4.53		

[a] Shoulder.

motivation for an alternate synthetic route originating from precursors other than preformed purines. The pyrimidine approach, *via* ring closure of appropriate 4-amino-5-glycosylaminopyrimidines, has already been found[12] to be unrewarding. This prompted us to investigate the possibility of utilizing imidazole nucleosides[13,14] as precursors of 7-glycosylpurines and we now wish to report[15] a new general synthetic route for the

(12) G. M. Blackburn and A. W. Johnson, *J. Chem. Soc.*, 4358 (1960).

(13) Glycosidation of imidazole derivatives *via* the mercury salt procedure followed by ring closure has previously produced 9-glycosylpurines as the major product: (a) J. Baddiley, J. G. Buchanan, F. E. Hardy, and J. Stewart, *J. Chem. Soc.*, 2893 (1959); (b) J. Baddiley, J. G. Buchanan and G. O. Osbourne, *ibid.*, 3606 (1958).

(14) L. B. Townsend, *Chem. Rev.*, 67, 533 (1967).

(15) A preliminary account of this work on the first glycosidation of an imidazole *via* the fusion procedure followed by ring closure to yield 7-β-D-ribofuranosyladenine has been reported: R. J. Rousseau, L. B. Townsend, and R. K. Robins, *Chem. Commun.*, 265 (1966).

Table II. R_f Values of Imidazole and Purine Nucleosides[a,b]

No.	Compound	Chromatographic solvent systems[c] A	B	C
IV	5-Bromo-4-nitro-1-(β-D-ribofuranosyl)-imidazole	0.73	0.65	0.82
XIII	5-Carboxamido-4-nitro-1-(β-D-ribofuranosyl)imidazole	0.80	0.31	0.67
XV	4-Amino-5-carboxamido-1-(β-D-ribofuranosyl)imidazole	0.76	0.14	0.53
III	Methyl 4-nitro-1-(β-D-ribofuranosyl)-imidazole-5-formimidate	0.80	0.53	0.82
IX	7-(β-D-Ribofuranosyl)adenine	0.51	0.20	0.57
VII	2-Methyl-7-(β-D-ribofuranosyl)adenine	0.63	0.28	0.66
XVII	7-(β-D-Ribofuranosyl)hypoxanthine	0.71	0.16	0.54
XIV	2-Methyl-7-(β-D-ribofuranosyl)-hypoxanthine	0.76	0.29	0.59
XVIII	7-(β-D-Ribofuranosyl)guanine	0.61	0.11	0.38

[a] All compounds were run on Whatman No. 1 chromatographic paper and descending technique was used. [b] Short-wave ultraviolet light (254 mμ) was used to detect spots. [c] Chromatographic solvent systems: A, 5% aqueous ammonium bicarbonate (w/w); B, 1-butanol saturated with water; C, 1-propanol–ammonium hydroxide (specific gravity 0.90)–water, 6:3:1 (v/v).

Table III. R_f Values of Acetylated Imidazole Nucleosides[a,b]

No.	Compound	Chromatographic solvent systems[c] 1	2	3	4
I	5-Bromo-4-nitro-1-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl)imidazole	0.77		0.35	0.93
	4-Bromo-5-nitro-1-(2′,3′,5′-tri-O-acetyl-D-ribofuranosyl)-imidazole	0.87		0.32	0.92
II	5-Cyano-4-nitro-1-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl)imidazole	0.78	0.58		0.94
V	5-Cyano-4-amino-1-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl)imidazole	0.65	0.63		0.82
XII	Methyl 4-nitro-1-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl)imidazole-5-carboxylate	0.80		0.33	0.86

[a] Chromatograms were developed on glass plates (5 × 20 cm) coated with a 250-μ layer of SilicAR-7GF by the ascending technique. [b] Spots were detected with a short-wave ultraviolet light (254 mμ). [c] Solvents: 1, 1-butanol saturated with water; 2, chloroform–ethanol, 20:1 (v/v); 3, chloroform–ethanol, 100:1 (v/v); 4, 1-butanol–glacial acetic acid–water, 6:3:1 (v/v).

preparation of 7-glycosylpurines from imidazole nucleosides prepared *via* the fusion procedure.[16,17]

A mixture of 4(5)-bromo-5(4)-nitroimidazole[18] and tetra-O-acetyl-β-D-ribofuranose was heated at 180–185° with chloroacetic acid to afford a 72% yield of 5-bromo-4-nitro-1-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl)imidazole (I). This was the only crystalline compound initially isolated from the fusion reaction, and although we expected this glycosidation reaction to produce a mixture of both N-substituted isomers, thin layer chromatography of this crystalline compound in four solvent systems showed the presence of only one isomer, The site of glycosidation was assigned from a comparison of the ultraviolet absorption spectra of I with ultraviolet absorption spectra reported[19] for 1-methyl-4-chloro-5-nitroimidazole and 1-methyl-5-chloro-4-nitroimidazole. The other isomer [4-bromo-5-nitro-1-(2′,3′,5′-tri-O-acetyl-D-ribofuranosyl)imidazole] was subsequently isolated in less than 5% yield from the ether filtrate and the structure was assigned on the basis of ultraviolet absorption spectral comparisons with model compounds,[19] *vide supra*. Deacetylation of I with liquid ammonia at room temperature furnished 5-bromo-4-nitro-1-(β-D-ribofuranosyl)imidazole (IV). However, it was subsequently observed that for nucleophilic displacement of the 5-bromo group it was more desirable to use the acetylated nucleoside I than the deacetylated nucleoside IV because of the difference in solubility characteristics and the increased strength of the glycosidic bond observed for the acetylated nucleoside I. A facile displacement of the 5-bromo group was observed, without cleavage of the glycosidic bond, when I was treated with potassium iodide and potassium cyanide in anhydrous dimethyl sulfoxide to afford an 82% yield of 5-cyano-4-nitro-1-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl)imidazole (II). This provided additional evidence for the site of glycosidation since bromine is readily displaced by cyanide only when the N-substituent resides adjacent to the bromo group.[20] Although the melting points of I (93–95°) and II (99–100°) are very close the difference between the two nucleosides can be readily ascertained by infrared spectra (II, 2250 cm^{-1} for C≡N), ultraviolet absorption spectra (Table I), chromatographic mobilities (Tables II and III), and elemental analyses.

Treatment of 5-cyano-4-nitro-1-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl)imidazole (II) with methanolic ammonia furnished a compound melting at 178–179° which we at first assumed to be the deacetylated nucleoside [5-cyano-4-nitro-1-(β-D-ribofuranosyl)imidazole]. However, an inspection of the infrared spectrum of this product revealed the absence of an absorption peak (2200–2300 cm^{-1}) for the cyano group. The proton magnetic resonance (pmr) spectrum in dimethyl sulfoxide-d_6 revealed a sharp singlet at δ 9.5 (one proton) and a sharp singlet at δ 3.9 (three protons) in addition to the absorption peaks which we had expected [the aromatic H-2 proton (δ 8.2) and the protons of the carbohydrate moiety (δ 3.5–6.0, nine protons)]. When D_2O was added to the dimethyl sulfoxide-d_6 solution there was observed a disappearance of the singlet at δ 9.5, and all absorption peaks attributable to the hydroxyl protons of the carbohydrate moiety, while the singlet (three protons) at δ 3.9 remained undisturbed. On the basis of the above data, the structure methyl 4-nitro-1-(β-D-ribofuranosyl)imidazole-5-formimidate (III) was assigned to the nucleoside with a melting point of 178–179° and this assignment was further corroborated by elemental analysis.

Catalytic reduction of the 4-nitro group of II was initiated using Raney nickel as a catalyst in a hydrogen atmosphere. This furnished a mixture of compounds and purification of this mixture furnished the desired compound [4-amino-5-cyano-1-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl)imidazole (V)] but with considerable

(16) R. J. Rousseau, L. B. Townsend, and R. K. Robins, *Biochemistry*, 5, 756, (1966); R. L. Tolman, R. K. Robins, and L. B. Townsend, *J. Heterocyclic Chem.*, 4, 230 (1967).

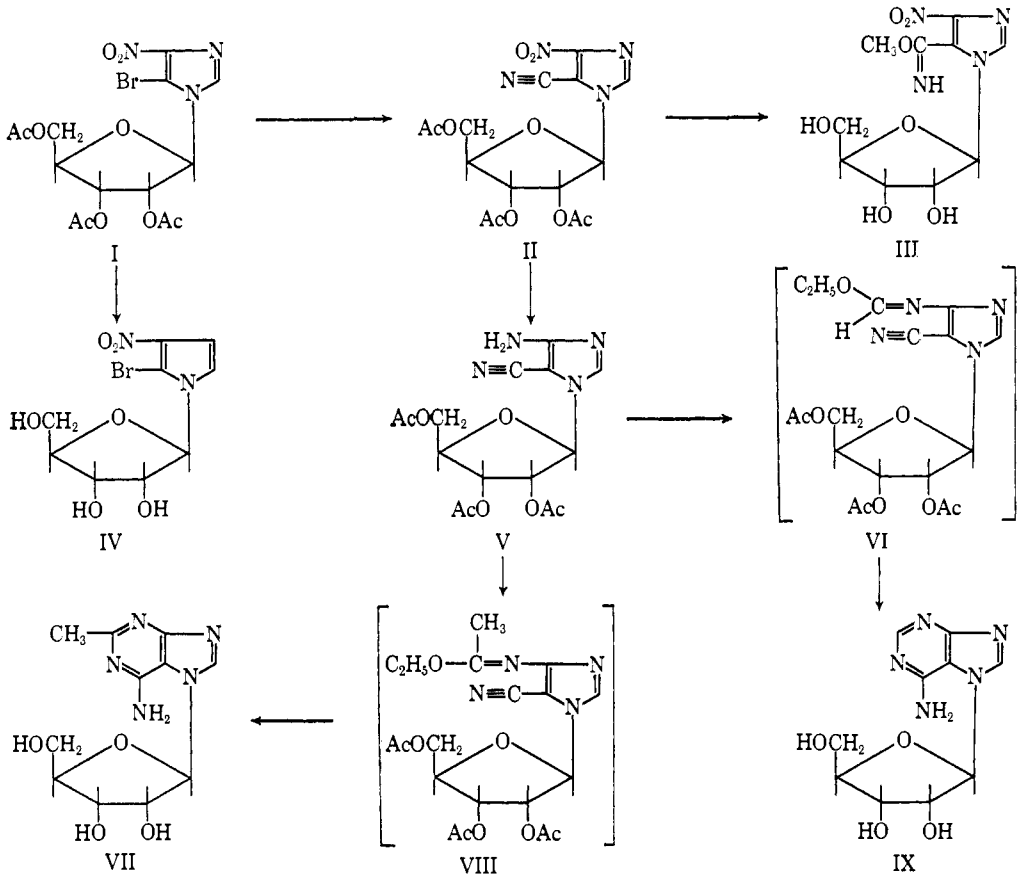
(17) The synthesis of 2-nitro-1-(β-D-ribofuranosyl)imidazole (azomycin riboside) has been recently reported using the fusion procedure: R. J. Rousseau, R. K. Robins, and L. B. Townsend, *J. Heterocyclic Chem.*, 4, 311 (1967).

(18) I. E. Balaban and F. L. Pyman, *J. Chem. Soc.*, 121, 947 (1922).

(19) G. C. Gallo, C. R. Pasqualucci, P. Radaelli, and G. C. Lancina, *J. Org. Chem.*, 29, 862 (1964).

(20) E. C. Taylor and P. K. Loeffler, *J. Am. Chem. Soc.*, 82, 3147 (1960).

Scheme I



loss of material. It was subsequently found that treatment of II in ethanol with 5% palladium on powdered charcoal catalyst in a hydrogen atmosphere afforded a colorless product in 87% yield which was chromatographically homogenous. The infrared absorption spectra of V revealed an absorption band at 2225 cm^{-1} (C≡N) which was very prominent. The pmr spectrum in $CDCl_3$ disclosed an absorption peak at δ 4.96 (two protons) which was assigned to the 4-amino group of 4-amino-5-cyano-1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole (V).

The reaction between V and diethoxymethyl acetate at reflux temperature was conveniently followed by a frequent chromatographic survey[21] of the reaction mixture. The 4-ethoxymethylene intermediate (VI) exhibits a larger R_f value than that observed for V (Table II) and the reaction was assumed to be complete when the ultraviolet absorbing spot attributed to V was no longer observed. Treatment of VI (syrup) with methanolic ammonia furnished 6-amino-7-(β-D-ribofuranosyl)purine (IX) in 93% yield. The ultraviolet absorption spectra (Table I), R_f values, melting point, and optical rotation observed for IX were found to be in accord with those previously reported[3] and firmly established the actual site of glycosidation and anomeric configuration for all nucleosides shown in Scheme I. Application of the $\Delta\lambda_{min}$ and $\Delta\delta$ rule[11,22] to IX provided additional substantiation for the assignment of N-7 as the site of glycosidation. The pmr spectrum revealed an absorption peak at δ 7.1 (two protons) which was assigned to the 6-amino group and would indicate that IX exists in the "amino" form,[23] in dimethyl sulfoxide-d_6.

It is of interest that 7-β-D-ribofuranosyladenine (IX) has recently been shown[24] to act as an excellent inhibitor of a certain specific nucleosidase which rapidly degrades adenosine.

A mixture of V and triethyl orthoacetate was heated at reflux temperature, in the presence of an acidic catalyst, to afford an orange syrup which was assumed to be the 4-ethoxyethylene derivative (VIII) of V. Treatment of VIII with liquid ammonia at room temperature resulted in a facile ring closure to afford 6-amino-2-methyl-7-(β-D-ribofuranosyl)purine (VII) as the monohydrate. The pmr spectrum of VII in dimethyl sulfoxide-d_6 exhibited absorption peaks at δ 9.6 (one proton), 7.0 (two protons), and 3.5 (three protons) which were assigned to the H-8 proton, 6-amino group, and 2-methyl group, respectively. This indicates that VII also exists in the "amino" form[23] in dimethyl sulfoxide-d_6. The ultraviolet spectral data observed for VII were very similar to the data reported for 2,7-dimethyladenine[25] and 2-methyl-7-ribosyladenine, which was isolated[26] from a naturally occurring analog of vitamin B_{12}. This provided additional proof that ring closure had indeed occurred.

The route we envisaged for the preparation of 7-(β-D-ribofuranosyl)purin-6-one and related derivatives[27] re-

(21) Micro thin-layer plates using SilicAR-7GF, Mallinckrodt Chemical Co., as absorbent and a mixture of benzene and ethanol as the solvent system.

(22) K. R. Darnall and L. B. Townsend, *J. Heterocyclic Chem.*, 3, 371 (1966).

(23) A full discussion of the pmr spectra of potentially tautomeric N-substituted adenines, *e.g.*, IX, will be the subject of a forthcoming joint contribution from the laboratory of Professor Nelson J. Leonard, University of Illinois and this laboratory.

(24) Private communication with Dr. E. T. Reese, U. S. Army Natick Laboratories, Natick, Mass.

(25) J. Davoll and B. A. Lowy, *J. Am. Chem. Soc.*, 74, 1563 (1952).

(26) W. Friedrich and K. Bernhauer, *Chem. Ber.*, 90, 465 (1957).

(27) A preliminary account of the work in this area has been reported:

quired the imidazole nucleoside 4-amino-5-carboxamido-1-(β-D-ribofuransoyl)imidazole. The preparation of this imidazole nucleoside has been previously reported[13a] to occur in low yield as part of an isomeric mixture, from a condensation of the silver salt of methyl 5(4)-nitroimidazole-4(5)-carboxylate[28] (X) and 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride after deblocking, chromatographic separation of isomers, and catalytic reduction. This prompted us to examine the possibility of utilizing the fusion procedure in an effort to increase the yield of the desired isomer. A mixture of methyl 5(4)-nitroimidazole-4(5)-carboxylate[27] (X) and tetra-*O*-acetyl-β-D-ribofuranose (XI) was heated at 170–175°, in the presence of an acidic catalyst, to afford a crystalline product in 83% yield. The structure of this nucleoside was established as methyl 4-nitro-1-(2,'-3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-5-carboxylate (XIII) on the basis of pmr and ultraviolet absportion spectra as well as the subsequent conversion of XII to a nucleoside (XVII) of established structure. We were unable to detect chromatographically any other isomer in the reaction mixture. Treatment of XII with methanolic ammonia furnished a 99% yield of crystalline nucleoside material. This nucleoside material was found to consist entirely of one isomer [5-carboxamido-4-nitro-1-(β-D-ribofuranosyl)imidazole (XIII)]. The structure was established by paper chromatography and pmr spectra, with additional corroboration provided by a comparison of melting point, ultraviolet absorption spectra, and specific rotation with the data previously reported[13a] for XIII.

Catalytic reduction (5% palladium on powdered charcoal) of the 4-nitro group of XIII was accomplished very smoothly to afford a 96% yield of 4-amino-5-carboxamido-1-(β-D-ribofuranosyl)imidazole (XV, iso-AICAR). The pmr spectra of XV exhibited the normal absorption peaks expected for the carbohydrate moiety and the aromatic proton (H-2), and in addition there was observed two sharp medium-sized singlets at δ 5.4 (two protons) and 6.9 (two protons) which were assigned to the two amino groups (4-amino and 5-carboxamido, respectively). For additional corroboration of the assignment of the above two absorption peaks, we examined the pmr spectra of XIII which possessed only the amino group of the carboxamido function. The absorption peak observed for the carboxamido group of XIII occurred at a lower field (δ 7.15–7.40) than the absorption peak for the carboxamido group of VX and substantiates the assignments made for XV.

This has now provided a new convenient synthesis of iso-AICAR and makes it available for the first time in sufficient quantity for extensive biological investigation. It is of interest that the biosynthetic pathway for the formation of 7-ribosylpurines isolated from the naturally occurring vitamin B_{12} analogs is as yet undetermined. It is tempting to postulate that there exists a biosynthetic pathway similar to the established[14] *de novo* pathway of purine biosynthesis, where 5-amino-4-carboxamido-1-(β-D-ribofuranosyl)imidazole-5-phosphate (AICAR) occurs as a central intermediate in the biosynthesis of 9-ribosylpurines. Thus it is possible that an intermediate similar to iso-AICAR (XV) could be utilized for the biosynthetic prepartion of 7-ribosylpurines. Treatment of XV with diethoxymethyl acetate at reflux temperature furnished 7-(β-D-ribofuranosyl)purin-6-one (XVII) in 77% yield and has provided the first synthesis of XVII of preparative value.[29] The ultraviolet absorption spectra of XVII was found to be very similar to that reported for 7-methylhypoxanthine and was found to be quite definitive when compared with the reported ultraviolet absorption spectral data for 1-methyl-,[30] 3-methyl-,[31] 7-methyl-,[32] and 9-methylhypoxanthine.[33]

Ring closure of XV with methylisothiocyanate in pyridine[34] proceeded very smoothly to afford 7-(β-D-ribofuranosyl)purin-6-one-2-thione (XVI) in excellent yield. The thione rather than the thiol form was indicated when there was observed an absorption band (very strong) in the infrared spectrum at 1560 cm^{-1} which was assigned[35,36] as C=S stretching and part of a —NC=S system. The thiol form was also eliminated by the absence of a band at 2550–2600 cm^{-1} usually attributable[36,37] to -SH stretching. The pmr spectra of XVI provided additional proof for this assignment when there was observed two absorption peaks, at δ 12.2 and 13.2 in dimethyl sulfoxide-d_6, which were assigned to protons resid ng at N-1 and N-3. Treatment of XVI with Raney nickel in water at reflux temperature provided another convenient route for the preparation of XVII. Deamination of 7-β-D-ribofuranosyladenine (IX) with nitrous acid afforded a compound which was shown to be identical in all respects (*e.g.*, optical rotations, ultraviolet absorption, infrared and pmr spectra, and a mixture melting point which showed no depression) with the compound prepared from ring closure of XV and dethiation of XV . Since the site of glycosidation and anomeric configuration of IX has been determined previously, *vide infra*, this established unequivocally the actual site of glycosidation and anomeric configuration of all nucleosides shown in Scheme II.

A facile ring closure of XV was effected with sodium ethoxide and ethyl acetate to furnish 2-methyl-7-β-D-ribofuranosylhypoxanthine (XIV). Deamination of VI with nitrous acid also furnished XIV in good yield.

The formation of 2-chloro-7-(β-D-ribofuranosyl)purin-6-one (XIX) was effected *in situ* on treatment of a suspension of XVI in methanol with chlorine gas at −40°.[38] Treatment of XIX with methanolic ammonia at 130° in a sealed vessel produced 7-β-D-ribofuranosylguanine (XVIII) in 85% yield which possessed ultraviolet pectral data in complete accord with the data reported for the 7-ribosylguaninephosphate isolated[39] from a naturally occurring vitamin B_{12} analog

R. J. Rousseau, R. K. Robins, and L. B. Townsend, First International Congress of Heterocyclic Chemistry, Albuquerque, N. M., 1967, Paper No. 2.

(28) L. P. Kuler and R. N. Givera, *Zh. Prikl. Khim.*, **30**, 811 (1957).

(29) Reference 8 reports 10 mg (6.7%); ref 9 reports a low yield from a mixture of compounds and ref 13a reported only ultraviolet absorption spectral data.

(30) L. B. Townsend and R. K. Robins, *J. Org. Chem.*, **27**, 990 (1962).

(31) F. Bergman, G. Levin, A. Kalmus, and H. Kwietny-Govrin, *ibid.*, **26**, 1504 (1961).

(32) R. N. Prasad and R. K. Robins, *J. Am. Chem. Soc.*, **79**, 6401 (1957).

(33) R. K. Robins and H. H. Lin., *ibid.*, **79**, 490 (1957).

(34) A. H. Cook and E. Smith, *J. Chem. Soc.*, 3001 (1949).

(35) R. M. Silverstein and G. C. Bassler, "Spectrometric Identification of Organic Compounds," 2nd ed, John Wiley and Sons, Inc., New York, N. Y., 1967, p 100.

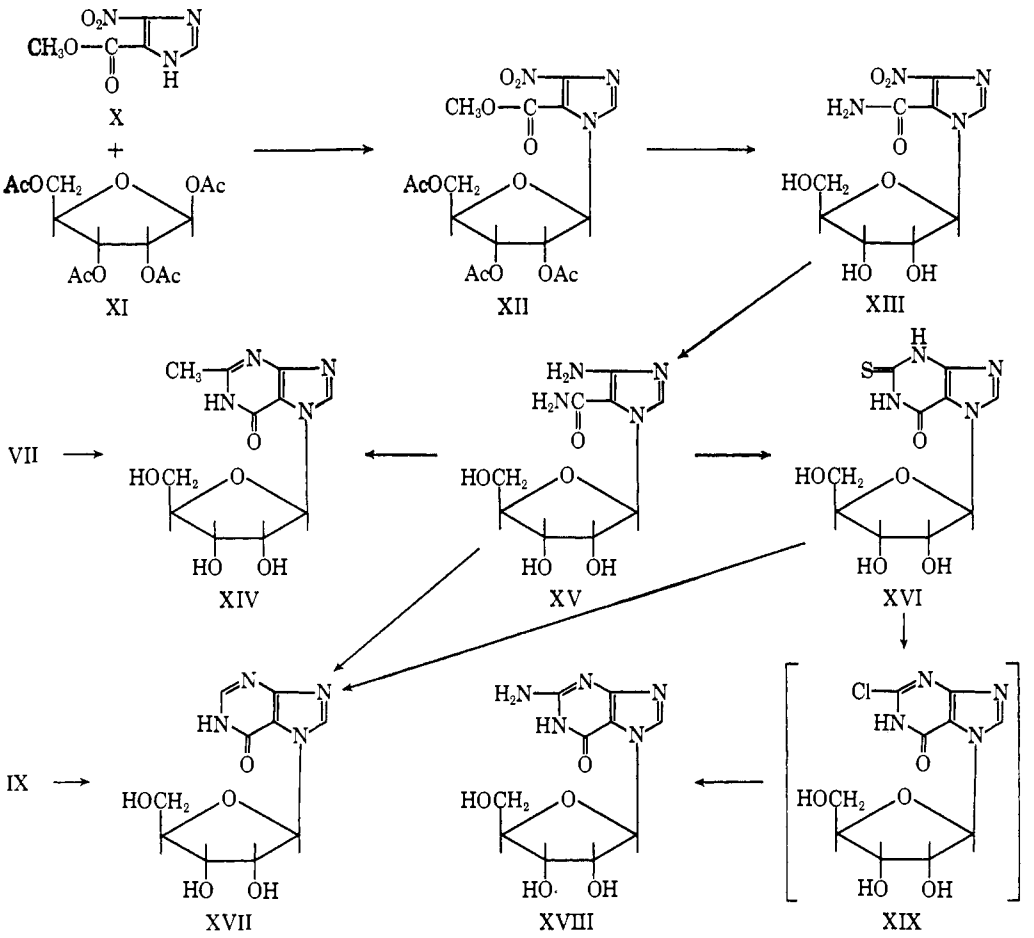
(36) D. Harrison and J. T. Ralph, *J. Chem. Soc., B*, 14 (1967).

(37) J. R. Dyer, "Applications of Absorption Spectroscopy of Organic Compounds," Prentice Hall Inc., Englewood Cliffs, N. J., 1965, p 38.

(38) R. K. Robins, *J. Am. Chem. Soc.*, **82**, 2654 (1960); R. K. Robins, *J. Org. Chem.*, **26**, 447 (1961).

(39) W. Friedrich and K. Bernhauer, *Z. Physiol. Chem.*, **317**, 116 (1959).

Scheme II



from digested sewage sludge. A comparison of the ultraviolet absorption spectral data of XVIII with 1-methyl-,[40] 3-methyl-,[41] 7-methyl-,[41] and 9-methylguanine[41] furnished additional proof of the above structure assignment. This would appear to be the preferred method for the preparation of 7-β-D-ribofuranosylguanine,[42] and in fact, for 7-glycosylguanines in general.[43]

A recent study[44] on the specificity of a certain nucleoside phosphorylase revealed that 7-glycosylpurines are not accepted as substrates for this phosphorylytic cleavage. Of considerable interest was the finding[44] that the addition of a 7-glycosylpurine to the reaction media reduced the rate of cleavage of thioguanosine (a riboside) while there was observed no effect on the rate of cleavage of 2′-deoxythioguanosine (a 2′-deoxyriboside) under similar conditions.

The preparation of additional 7-glycosylpurines *via* the general route described herein utilizing imidazole nucleosides as precursors is under active investigation in this laboratory.

Experimental Section[45]

Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. The nuclear magnetic resonance spectra were obtained on a Varian A-60 high-resolution nmr spectrometer utilizing tetramethylsilane as an internal standard, ultraviolet spectra with a Cary 14 ultraviolet spectrometer, optical rotations with a Rudolph polarimeter, and infrared spectra with a Beckmann IR-5A spectrometer.

5-Bromo-4-nitro-1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole (I). A dry, finely powdered mixture of 4(5)-bromo-5(4)-nitroimidazole[18] (10.0 g) and 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (18.0 g) in a 100-ml, pear-shaped flask was immersed in an oil bath which had been preheated to 205–215°. Chloroacetic acid (0.5 g) was added when the reaction mixture had reached 160°. Boiling occurred when the reaction mixture temperature reached 182° and a vacuum (0.10 mm) was applied for 10 min. The mixture was then removed from the oil bath and triturated while hot with 200 ml of benzene. Three additional 10-g (imidazole) fusions were repeated by the above procedure. The combined mixtures (800 ml of benzene) were heated to boiling and the solid was collected by filtration and washed with boiling benzene (50 ml) to furnish 4.8 g of 4(5)-bromo-5(4)-nitroimidazole. The benzene solution including washings was extracted with cold saturated aqueous sodium carbonate solution (four 100-ml portions) and water (two 100-ml portions) and dried over anhydrous magnesium sulfate. After the drying agent was removed by filtration the benzene solution was evaporated to a syrup *in vacuo* and triturated with 400 ml of diethyl ether. The colorless crystals were collected and washed with diethyl ether to give 56.5 g (72% based on unrecovered starting material). Recrystallization from ethanol afforded heavy crystals, mp 92–95°, $[\alpha]^{25}D$ +1.71° (*c* 1, $CHCl_3$).

Anal. Calcd for $C_{14}H_{16}BrN_3O_9$: C, 37.40; H, 3.56; N, 9.34; Br, 17.70. Found: C, 37.25; H, 3.37; N, 9.21; Br, 17.90.

4-Bromo-5-nitro-1-(2′,3′,5′-tri-*O*-acetyl-D-ribofuranosyl)imidazole. The ether filtrate from the above procedure (four fusions) was allowed to evaporate to dryness at room temperature and pressure. The residual syrup was dissolved in ethanol and allowed to evaporate to dryness again at room temperature and pressure to

(40) W. Pfleiderer, *Ann. Chem.*, 647 167 (1961).
(41) L. B. Townsend and R. K. Robins, *J. Am. Chem. Soc.*, 84, 3008 (1962).
(42) K. Imai, A. Nohara, and M. Honjo, *Chem. Pharm. Bull.* (Tokyo), 14, 1377 (1966), have reported XVIII as one component of a mixture of four compounds (isomeric and anomeric).
(43) Z. A. Shabarova, Z. P. Polyakova, and M. A. Prokafev, *Zh. Obshch. Khim.*, 29, 215 (1959).
(44) G. A. Lepage and I. G. Junga, *Cancer Res.*, 25, 46 (1965).

(45) The authors are indebted to Mr. A. F. Lewis and Mr. S. M. Reddick for the large-scale preparation of various imidazole and imidazole nucleoside intermediates.

provide a crystalline substance (2.2 g) which was triturated in ethanol, collected by filtration and then boiled in 25 ml of ethanol. The solid which remained out of solution was collected by filtration and recrystallized from 250 ml of ethanol (anhydrous) to afford shiny crystals (2.0 g), mp 192–193°, $[\alpha]^{27}D$ −109.0° (*c* 1, $CHCl_3$).

Anal. Calcd for $C_{14}H_{16}BrN_3O_9$: C, 37.40; H, 3.56; N, 9.34. Found: C, 37.60; H, 3.70; N, 9.30.

5-Bromo-4-nitro-1-(β-D-ribofuranosyl)imidazole (IV). Deacetylation of 5-bromo-4-nitro-1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-imidazole (I, 5.3 g) with 100 ml of liquid ammonia at 20° for 4 hr was accomplished in a steel reaction vessel. The ammonia was removed at room temperature and the residual ammonia removed *in vacuo* at room temperature. The syrup was triturated with 50 ml of anhydrous ethanol and then allowed to stand at 5° for 18 hr, yield 3.8 g (quantitative). This crystalline material was recrystallized from water to afford a nucleoside with a melting point of 174–175° and $[\alpha]^{24}D$ −8.54° (*c* 1, H_2O).

Anal. Calcd for $C_8H_{10}BrN_3O_6$: C, 29.60; H, 3.09; N, 12.95. Found: C, 29.80; H, 3.15; N, 13.08.

5-Cyano-4-nitro-1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole (II). To a mixture of 6.6 g of potassium cyanide and 0.6 g of potassium iodide in 200 ml of dimethyl sulfoxide was added 20.0 g of 5-bromo-4-nitro-1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole (I). The mixture was stirred at room temperature, under anhydrous conditions for 16 hr and then poured into 800 ml of ice water. The resulting aqueous solution was extracted with methylene chloride (five 100-ml portions). The methylene chloride layer was then extracted with water (four 100-ml portions), dried over anhydrous magnesium sulfate, and evaporated *in vacuo* to a brown syrup. The syrup was triturated at room temperature with 200 ml of ethanol (anhydrous) until complete crystallization occurred. The mixture was allowed to stand at room temperature overnight and the product collected by filtration and recrystallized from 450 ml of ethanol to give colorless needles, yield 15 g (84%), mp 99–101°, $[\alpha]^{23}D$ +11.2° (*c* 1, $CHCl_3$).

Anal. Calcd for $C_{15}H_{16}N_4O_9$: C, 45.46; H, 4.07; N, 14.14. Found: C, 45.40; H, 4.16; N, 14.24.

Methyl 4-Nitro-1-(β-D-ribofuranosyl)imidazole-5-formimidate (III). 5-Cyano-4-nitro-1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-imidazole (II, 10 g) was treated with 200 ml of saturated methanolic ammonia (previously saturated at 0°) at 0° for 48 hr. The solution was evaporated to a glass *in vacuo* and then dissolved in a small amount of ethanol and allowed to stand at 5° for 18 hr. The product which had separated was collected by filtration and washed with ethanol (anhydrous) to yield 1.6 g of a solid material. This solid was recrystallized from water to yield long thin colorless needles, mp 178–179° $[\alpha]^{23}D$ −11.97° (*c* 1, H_2O).

Anal. Calcd for $C_{10}H_{14}N_4O_7$: C, 39.74; H, 4.67; N, 18.54. Found: C, 39.50; H, 4.71; N, 18.60.

4-Amino-5-cyano-1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole (V). Ten grams of 5-cyano-4-nitro-1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole (II) was dissolved in 400 ml of anhydrous ethanol and slowly warmed until a clear solution was effected. The solution was transferred to a 2-l. hydrogenation bottle, purged with nitrogen, and 5.0 g of 5% palladium on charcoal was carefully added. The mixture was hydrogenated with shaking in a Paar hydrogenator at 50 psi. The pressure after 1 hr was 45 psi, and the reaction mixture was allowed to remain on the hydrogenator for an additional hour (final pressure 45 psi). The catalyst was collected by filtration and the filtrate evaporated to a solid foam *in vacuo* to yield 8.0 g (87.4%), mp 58–60°, $[\alpha]^{23}D$ −10.9° (*c* 1, $CHCl_3$).

Anal. Calcd for $C_{15}H_{18}N_4O_7$: C, 49.18; H, 4.95; N, 15.29. Found: C, 49.22; H, 4.91; N, 15.20.

6-Amino-7-(β-D-ribofuranosyl)purine (IX, 7-β-D-Ribofuranosyladenine). 4-Amino-5-cyano-1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole (V, 2.8 g) was heated at reflux temperature in 28 ml of diethoxymethyl acetate[46,47] for 2.5 hr. The orange solution was evaporated *in vacuo* to a syrup, the syrup was dissolved in toluene (50 ml) and again evaporated *in vacuo* to a syrup. This process was repeated once more and the residual syrup was dissolved in 250 ml of methanolic ammonia (previously saturated at 0°). The solution was evaporated to dryness *in vacuo* and triturated with a small amount of anhydrous ethanol. The colorless precipitate was collected by filtration to yield 1.9 g of product. The solid was recrystallized from water for analysis, mp 243–244°, $[\alpha]^{27}D$ −85.1° (*c* 0.35, H_2O) [lit.[3] $[\alpha]^{25}D$ −84.9 ± 0.2° (*c* 0.35, H_2O)].

Anal. Calcd for $C_{10}H_{13}N_5O_4$: C, 44.94; H, 4.90; N, 26.21. Found: C, 45.17; H, 4.99; N, 25.92.

6-Amino-2-methyl-7-(β-D-ribofuranosyl)purine (VII, 2-Methyl-7-β-D-ribofuranosyladenine). 4-Amino-5-cyano-1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole (V, 2.0 g) was heated at reflux temperature in 20 ml of triethyl orthoacetate and 0.5 ml of glacial acetic acid for 2.5 hr. The orange solution was evaporated to a syrup *in vacuo*, dissolved in 100 ml of liquid ammonia and the reaction mixture allowed to stand at room temperature for 16 hr in a stainless steel reaction vessel. The reaction mixture was then evaporated to a semisolid *in vacuo* and triturated with chloroform (three 50-ml portions) at room temperature. The resulting light tan powder was collected to yield 1.53 g of product. This solid was recrystallized from 20 ml of ethanol to which a small amount of water had been added to afford complete solution. The colorless, heavy, transparent crystals were collected by filtration, mp 161° (bubbles), $[\alpha]^{27}D$ −85.7° (*c* 1, H_2O).

Anal. Calcd for $C_{11}H_{15}N_5O_4 \cdot H_2O$: C, 44.15; H, 5.73; N, 23.40. Found: C, 44.40; H, 5.89; N, 23.40.

2-Methyl-7-(β-D-ribofuranosyl)purin-6-one (XIV, 2-Methyl-7-β-D-ribofuranosylhypoxanthine). Method A. 4-Amino-5-carboxamido-1-(β-D-ribofuranosyl)imidazole (XV, 1.0 g) and 2.8 g of ethyl acetate were added to a stirred solution of sodium ethoxide (prepared by dissolving 0.8 g of sodium metal in 20 ml of anhydrous ethanol). This solution was heated at reflux temperature for 6 hr, then diluted with 50 ml of water and the pH of the solution adjusted to 3 with Amberlite IR-120H. The resin was collected by filtration and washed thoroughly with water and the filtrate evaporated to a glass *in vacuo*. The glass was dissolved in 10 ml of water and treated with charcoal, the charcoal removed, and the filtrate concentrated to *ca.* 3 ml. Acetone (12 ml) was then added, and the mixture was allowed to cool for 6 hr at 5°. The shiny crystals (500 mg) were then collected by filtration (mp 220–222°). An analytical sample was prepared by the elution of a small amount of the above product from a dry-packed cellulose column with a methanol–water (7:3, v:v) solution. The uv-absorbing fractions were evaporated to dryness *in vacuo* and the resulting residue dissolved in a small amount of water and lyophylized to afford an analytical sample, mp 232–234° dec.

Anal. Calcd for $C_{11}H_{14}N_4O_5 \cdot H_2O$: C, 44.00; H, 5.37; N, 18.66. Found: C, 43.88; H, 4.99; N, 18.71.

Method B. The same reaction conditions were utilized for the preparation of XIV from XV as those used for the preparation of IX (method B). This furnished a 60% yield of crude XIV which was recrystallized two times from ethanol. The product was identical in all respects with the product obtained from method A.

Methyl 4-Nitro-1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-5-carboxylate (XII). Methyl 5(4)-nitroimidazole-4(5)carboxylate[28] (10.0 g) and 20 g of tetra-*O*-acetyl-β-D-ribofuranose were placed in an oil bath which had been preheated to 190°. When the temperature of the reaction mixture had reached 175° a colorless homogeneous melt was observed and approximately 0.5 g of chloroacetic acid was then added. At 178° rapid bubbling occurred and then a vacuum (0.1 mm) was applied for 10 min. The residue was triturated with 200 ml of benzene and 3.6 g of the unreacted imidazole was collected. The benzene solution was then extracted with cold saturated aqueous Na_2CO_3 (four 50-ml portions) solution followed by water (two 50-ml portions), dried over anhydrous $MgSO_4$, and then allowed to stand overnight at room temperature to afford 3.7 g of light yellow crystals, mp 113–118°. The filtrate was evaporated to a semisolid *in vacuo* and triturated with 50 ml of anhydrous ethanol and 7.4 g of colorless product (mp 110–116°) was collected. The ethanol filtrate provided an additional 2.3 g of crystalline product (mp 112–117°) after remaining overnight at room temperature. Combined total yield of 13.4 g, 83% based on unrecovered starting material. This was recrystallized from ethanol to afford light yellow transparent needles, mp 116–118°, $[\alpha]^{28}D$ +29.5° (*c* 1, $CHCl_3$).

Anal. Calcd for $C_{16}H_{19}N_3O_{11}$: C, 44.75; H, 4.44; N, 9.79. Found: C, 44.39; H, 4.35; N, 9.79.

5-Carboxamido-4-nitro-1-(β-D-ribofuranosyl)imidazole (XIII). Methyl 4-nitro-1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-5-carboxylate (5 g) was added to methanolic ammonia (250 ml) (saturated at 0°) and stirred for 16 hr at room temperature. The solution was then evaporated *in vacuo* to a solid which was triturated in 50 ml of anhydrous ethanol to furnish 3.3 g (99%) of the desired product, mp 177–178°. This product was recrystallized from an ethanol–water mixture to give colorless needles (dried at 80°), mp

(46) H. W. Post and E. R. Erickson, *J. Org. Chem.*, **2**, 260 (1937).

(47) We have recently received a modified preparation of diethoxymethyl acetate, private communication from Dr. J. A. Montgomery.

177–178° (lit.[13a] 178°), $[\alpha]^{20}D$ −3.00° (*c* 1.0, H_2O) [lit.[13a] $[\alpha]D$ −0.60° (*c* 1.02, H_2O)].

Anal. Calcd for $C_9H_{12}N_4O_7$: C, 37.51; H, 4.20; N, 19.44. Found: C, 37.49; H, 4.05; N, 19.35.

4-Amino-5-carboxamido-1-(β-D-ribofuranosyl)imidazole (XV). 5-Carboxamido-4-nitro-1-(β-D-ribofuranosyl)imidazole (XIII, 5.0 g) was dissolved in methanol (100 ml), purged with nitrogen before the catalyst (1.5 g of 5% palladium on charcoal) was added, and hydrogenated in a Parr hydrogenator with shaking at 50 psi for 3 hr. The catalyst was removed by filtration and washed with methanol, and the filtrate and washings were combined and evaporated *in vacuo* to a colorless powder (4.3 g, 96%), mp 180–184°. This solid was dissolved in a small amount of methanol and allowed to stand at 5°, for 18 hr to yield white crystals, mp 186–187° (lit.[11] 187–189°), $[\alpha]^{26}D$ −30.9° (*c* 1, H_2O).

Anal. Calcd for $C_9H_{14}N_4O_5$: C, 41.86; H, 5.46; N, 21.70. Found: C, 41.99; H, 5.62; N, 21.75.

7-(β-D-Ribofuranosyl)purin-6-one (XVII, 7-β-D-Ribofuranosylhypoxanthine). Method A. 7-(β-D-Ribofuranosyl)purin-6-one-2-thione (XVI, 500 mg) was dissolved in 25 ml of boiling water. Raney nickel[48] (4.0 g) was then added and the reaction mixture heated at reflux temperature for 5 hr. The Raney nickel was removed by filtration and washed thoroughly with boiling water. The combined filtrate and washings were evaporated to dryness *in vacuo* and the solid was triturated with methanol and collected by filtration to yield 200 mg of product. This solid was recrystallized from methanol by slow evaporation to furnish the desired product, mp 229–230°, $[\alpha]^{26}D$ +15.9° (*c* 1, 0.1 *N* NaOH).

Anal. Calcd for $C_{10}H_{12}N_4O_5$: C, 44.78; H, 4.51; N, 20.89. Found: C, 44.71; H, 4.61; N, 20.69.

Method B. 6-Amino-7-(β-D-ribofuranosyl)purine (IX, 500 mg) and sodium nitrite (400 mg) were dissolved in 25 ml of water. To this solution was slowly added 7.5 ml of 1.0 *N* HCl while maintaining the pH at 4–5 (the flask was loosely stoppered). After all the solid had dissolved, an additional 400 mg of sodium nitrite was added, and the loosely stoppered flask containing the reaction solution was allowed to stand at room temperature for 48 hr. The stopper was then removed from the flask and the reaction mixture allowed to stand at room temperature for 18 hr. The resulting colorless solution was evaporated to dryness *in vacuo*, the solid residue was extracted with a small amount of boiling methanol (20 ml), and the methanol was decanted from the residual solid material and allowed to slowly evaporate to dryness in a petri dish at room temperature. The clusters of crystals (300 mg) were recrystallized from methanol to give clusters of shiny crystals, mp 227–229°. Mixture melting point with the product obtained by method A showed no depression, $[\alpha]^{27}D$ +14.8° (*c* 1, 0.1 *N* NaOH).

(48) Purchased from W. R. Grace and Co., South Pittsburgh, Tenn.

Method C. 4-Amino-5-carboxamido-1-(β-D-ribofuranosyl)-imidazole (XV, 500 mg) was heated at reflux temperature in 10 ml of diethoxymethyl acetate[46,47] for 4 hr. The orange solution was evaporated *in vacuo* and the resulting amber syrup was dissolved in methanolic ammonia (250 ml) (saturated at 0°) and allowed to stand in a sealed vessel at room temperature for 24 hr. The light brown solution was evaporated to a glass *in vacuo* and triturated with acetone (200 ml), and the remaining light tan solid was collected by filtration to yield 400 mg of product. This solid was dissolved in hot methanol and allowed to evaporate slowly to dryness in a petri dish to provide clusters of tan crystals. Recrystallization from methanol gave crystals with a melting point of 228–330°. This compound was identical in all respects with the products obtained from methods A and B.

7-(β-D-Ribofuranosyl)purin-6-one-2-thione (XVI, 7-β-D-Ribofuranosylhypoxanthine-2-thione). 4-Amino-5-carboxamido-1-(β-D-ribofuranosyl)imidazole (XV, 1.0 g) was heated at reflux temperature in a mixture of pyridine (50 ml) and methylisothiocyanate (2 ml) for 16 hr. The solution was evaporated to a syrup *in vacuo*, dissolved in benzene, and again evaporated to dryness *in vacuo;* this process was repeated three times. The solid residue was then heated in 150 ml of benzene to reflux temperature and the solid collected by filtration to yield 1.8 g of a light tan solid. Two recrystallizations from water gave tan crystals (1.0 g) which darkened at 218–220° and decomposed with bubbling at 226°, $[\alpha]^{26}D$ −4.6° (*c* 1, 0.1 *N* NaOH).

Anal. Calcd for $C_{10}H_{12}N_4O_5S \cdot 0.5H_2O$: C, 38.90; H, 4.21; N, 18.15. Found: C, 38.90; H, 4.08; N, 18.18.

2-Amino-7-(β-D-ribofuranosyl)purin-6-one (XVIII, 7-β-D-Ribofuranosylguanine). 7-β-D-Ribofuranosylhypoxanthine-2-thione (XVI, 500 mg) was dried at 100° for 2 hr and then suspended in 3 ml of anhydrous methyl alcohol. The mixture was cooled in an acetone–Dry Ice bath at −40° and chlorine gas passed into the mixture (keeping the temperature below −30°) until all the solid material had dissolved (approximately 15 min). Air was then bubbled through until the solution was nearly colorless while the bath temperature was maintained at −30° (approximately 30 min). This solution was added slowly (dropwise) to 200 ml of methanol (previously saturated with ammonia at 0°) in a glass reaction vessel liner. The solution was heated at 130° in a stainless steel reaction vessel for 5 hr and then allowed to cool to room temperature. The reaction mixture was evaporated to a light tan solid *in vacuo* and triturated with 100 ml of methanol at room temperature and the solid collected by filtration and washed with methanol (200 ml) to yield 400 mg (85%) of the desired product. Recrystallization from water furnished an analytical sample which darkened at 230–260° and turned black at 263°, $[\alpha]^{26}D$ −11.75° (*c* 0.6, 0.1 *N* NaOH).

Anal. Calcd for $C_{10}H_{13}N_5O_5 \cdot H_2O$: C, 39.87; H, 5.02; N, 23.25. Found: C, 39.72; H, 4.88; N, 23.75.