Synthesis of Pyridazine Analogues of the Naturally Occurring Nucleosides Cytidine, Uridine, Deoxycytidine, and Deoxyuridine

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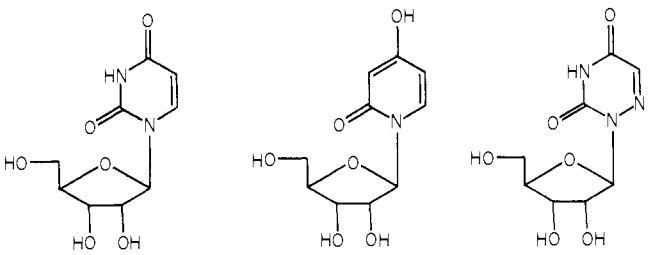
The condensation of 4,5-dichloro-3-[(trimethylsilyl)oxy]pyridazine (5) with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (6) was accomplished by the stannic chloride procedure to yield 4,5-dichloro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyridazin-6-one (7). Procedures used to unequivocally determine the site of ribosylation and anomeric configuration of 7 are discussed. Treatment of 7 with liquid ammonia effected a concomitant removal of the blocking groups and selective nucleophilic displacement of the 4-chloro group. Subsequent dehalogenation yielded 4-amino-1-(β-D-ribofuranosyl)pyridazin-6-one (11, 6-aza-3-deazacytidine). Treatment of 7 with methanolic sodium methoxide, followed by dehalogenation and hydrolysis with aqueous alkali, yielded 4-hydroxy-1-β-D-ribofuranosylpyridazin-6-one (6-aza-3-deazauridine, 15). The syntheses of various nucleosides derived from 7, 11, and 15 are described. Condensation of 5 with 3,5-di-*O*-*p*-toluoyl-2-deoxy-D-*erythro*-pentofuranosyl chloride (27) gave a mixture of the blocked anomeric 2′-deoxynucleosides 28 and 29. Nucleoside 28, the β anomer, was treated in the same manner as 7 to yield 4-amino-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyridazin-6-one (32, 6-aza-3-deaza-2′-deoxycytidine) and 4-hydroxy-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyridazin-6-one (34, 6-aza-3-deaza-2′-deoxyuridine). 6-Aza-3-deazauridine (15) was found to inhibit the growth of L1210 cells with an ID_{50} of about 7×10^{-5} M.

The interchange of nitrogen and carbon atoms in the heterocyclic base moieties of the primary nucleosides found in RNA and DNA has been one of the more successful methods in the development of nucleoside antimetabolites.[1,2] For example, investigations involving the design and synthesis of isosteric analogs of uridine (1) have led



1 2 3

to the synthesis of the biologically active nucleosides 3-deazauridine (2) and 6-azauridine (3). 3-Deazauridine,[3] a molecule in which a carbon atom has been substituted for the nitrogen atom in the 3-position of uridine, is a substrate for uridine kinase and is converted to its triphosphate nucleotide,[4] which exerts its action through an inhibition of the pyrimidine de novo enzyme, cytidine triphosphate synthetase.[5,6] 3-Deazauridine has been found active both in vitro[7] and in vivo[8] as an antibacterial and as an antitumor and antiviral agent.[9-11] 6-Azauridine,[12,13] an isostere of uridine containing a nitrogen atom in the 6-position, is also phosphorylated by uridine kinase to the biologically active 6-azauridine 5′-phosphate, which then acts as a competitive inhibitor of orotidine 5′-phosphate decarboxylase.[14-16] As part of a program to develop inhibitors of enzymes involved in the de novo synthesis of pyrimidine nucleosides and nucleotides, we have prepared the pyridazine nucleoside derivatives of uridine,[17] cytidine,[17] 2′-deoxyuridine, and 2′-deoxycytidine. This study was undertaken to ascertain if a molecule which possesses a combination of the skeletal modifications of both 3-deazauridine and 6-azauridine would be biologically active, mimic the mode of action of either of these two nucleosides, demonstrate an activity which is a combination of both modes of inhibition demonstrated by 2 and 3, or perhaps possess a mode of action which is unique to itself.

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Chemistry. We elected to use 4,5-dichloropyridazin-6-one (4; Scheme I) as our starting material, not only because of its ready availability by a simple synthetic route[18] but also because of the already reported susceptibility of the chloro groups toward a variety of nucleophilic substitution reactions.[19] Silylation of 4 was accomplished using hexamethyldisilazane, with a catalytic amount of ammonium sulfate, to yield 4,5-dichloro-3-[(trimethylsilyl)oxy]pyridazine (5), which was used in the subsequent reaction without purification. The silyl derivative was condensed with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (6) by a stannic chloride catalyzed silyl procedure[20,21] to furnish an 88% yield of nucleoside material, which was assigned the structure 4,5-dichloro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyridazin-6-one (7) on the

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Table I. ^{1}H NMR Spectral Data for Some Pyridazine Nucleosides

no.	solvent[a]	H1'	$J_{1',2'}$, Hz	others[b]	no.	solvent[a]	H1'	$J_{1',2'}$, Hz	others[b]
7	D	6.78 (d)	2	8.23 (s, H_5), 8.1 (m, Bz H's)	25	D	6.24 (d)	3	7.99 (s, H_3)
8	D	6.75 (d)	1	7.03 (d, H_5, $J_{4,5}$ = 9)					2.68 (s, SCH_3)
9	D			8.11 (dd, H_3, $J_{3,4}$ = 3.7)					2.59 (s, SCH_3)
				7.53 (dd, H_4, $J_{4,5}$ = 9.5)	26	D	6.24 (d)	3	7.90 (s, H_3)
				7.04 (dd, H_5, $J_{5,3}$ = 1.6)					7.33 (s, Bzl Ar H's)
10	D	6.22 (d)	3.5	7.80 (s, H_3)					7.26 (s, Bzl Ar H's)
				7.00 (br s, NH_2)					6.40 (s, Bzl CH_2)
11	D	6.19 (d)	4	7.60 (d, H_3, $J_{3,5}$ = 2.5)	28	C	6.93 (t)	6	7.80–8.00 (m, ortho H's toluoyl)
				6.62 (br s, NH_2)					7.62 (s, H_3)
				5.60 (d, H_5)					7.10–7.30 (m, meta H's, toluoyl)
12	D	6.36 (d)	1	7.73 (s, H_3)					5.78 (m, H_3')
				7.00 (br s, NH_2)					4.54 (m, H_4' + H_5')
				1.33 (s, CH_3)					2.50–3.20 (m, H_2')
				1.52 (s, CH_3)					2.40 (s, Ar CH_3)
13	D	6.30 (d)	3.5	8.45 (s, H_3)	29	C	6.67 (dd)	7, 3	7.60–7.80 (m, ortho H's, toluoyl)
				4.17 (s, OCH_3)					7.00–7.20 (m, H's, toluoyl)
14	D	6.21 (d)	4	7.85 (d, H_3, $J_{3,5}$ = 3)					5.43 (m, H_3')
				6.29 (d, H_5)					4.86 (m, H_4')
				3.88 (s, OCH_3)					4.50 (m, H_5')
15	D	6.22 (d)	3.5	7.92 (d, H_3, $J_{3,5}$ = 2.5)					2.30 (s, Ar CH_3)
				6.10 (d, H_5)	30	D	6.59 (t)	6	7.66 (s, H_3)
18	D	6.38 (d)	3	7.64 (d, H_3, $J_{3,5}$ = 3)					6.74 (br s, NH_2)
				6.67 (br s, NH_2)					5.07 (d, C_3', OH, J = 5)
				5.53 (d, H_5)					4.50 (t, C_5', OH, J = 5)
				2.08–2.14 (Ac Me H's)					4.31 (m, H_3')
19	C	6.36 (d)	3	7.80 (d, H_3, $J_{3,5}$ = 3)					3.74 (m, H_4')
				5.99 (d, H_5)					3.42 (m, H_5')
				2.04–2.13 (Ac Me H's)	31	D	6.67 (t)	6	8.29 (s, H_3)
20	D	6.22 (d)	3	7.97 (s, H_3)					4.11 (s, OCH_3)
				6.79 (br q, NH)	32	D	6.50 (t)	6	7.50 (d, H_3, $J_{3,5}$ = 3)
				2.99 (d, NCH_3, J = 5)					6.50 (br s, NH_2)
21	D	6.12 (d)	4	7.51 (d, H_3, $J_{3,5}$ = 2)					5.48 (d, H_5)
				7.10 (br q, NH)	33	D	6.60 (t)	6	7.82 (d, H_3, $J_{3,5}$ = 3)
				5.34 (d, H_5)					6.23 (d, H_5)
				2.69 (d, NCH_3, J = 5)	34	D	6.54 (t)	6	7.74 (d, H_3, $J_{3,5}$ = 3)
22	D	6.22 (d)	3	7.80 (s, H_3)					6.04 (d, H_5)
				2.94 [s, $N(CH_3)_2$]					
				2.68 [s, $N(CH_3)_2$]					
23	D	6.21 (d)	4	7.97 (s, H_3)					
				3.21 [s, $N(CH_3)_2$]					
24	D	6.18 (d)	4	7.93 (d, H_3, $J_{3,5}$ = 3)					
				5.52 (d, H_5)					
				3.01 [s, $N(CH_3)_2$]					

[a] D = dimethyl-d_6 sulfoxide; C = chloroform-d_6. [b] Coupling constant (J) in hertz. Abbreviations used: Bz, benzoyl; Bzl, benzyl; Ac, acetyl; Ar, aromatic.

basis of subsequent studies. Dehalogenation of **7** furnished 1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyridazin-6-one (**8**) identical with the compound previously reported.[22] In addition, removal of the blocking groups from compound **8** with methanolic sodium methoxide furnished a nucleoside which was identical with the previously reported 1-β-D-ribofuranosylpyridazin-6-one (**9**).[23] The ^{1}H NMR spectrum of **9** consists of three signals in the aromatic region [δ 8.11 (dd, 1 H) for H_3, exhibiting a coupling with H_4 of $J_{3,4}$ = 3.7 Hz and a small coupling to H_5 of 1.6 Hz; δ 7.53 (dd, 1 H) for H_4, $J_{4,5}$ = 9.5 Hz and $J_{4,3}$ = 3.7 Hz; δ 7.04 (dd, 1 H) for H_5, $J_{4,5}$ = 9.5 Hz and $J_{5,3}$ = 1.6 Hz], characteristic of unsubstituted 1-alkylpyridazin-6-ones.[24] If one considers the coupling constants for the aromatic protons, the ^{1}H NMR analysis is diagnostic for all substituted N-1 substituted pyridazin-6-one compounds (chemical shifts and assignments are summarized in Table I). In previous studies,[22] the authors provided no proof for assigning the site of ribosylation as N-1 of the heterocycle and based their assignment of anomeric configuration solely on the "trans rule".[25] Studies were therefore undertaken in our laboratory to confirm these assignments.

Treatment of nucleoside **7** with liquid ammonia at 110 °C removed the benzoyl blocking groups and effected a simultaneous nucleophilic displacement of the 4-chloro group to yield 4-amino-5-chloro-1-β-D-ribofuranosylpyridazin-6-one (**10**). Prolonged treatment of **7** with liquid ammonia (2–4 days at 110 °C) did not result in a displacement of the remaining chloro group. Treatment of **10** with hydrogen (40 psi) in the presence of 10% palladium on carbon catalyst afforded a 60% yield of the desired 4-amino-1-β-D-ribofuranosylpyridazin-6-one (**11**, 6-aza-3-deazacytidine). The ultraviolet spectral data for **11** was essentially identical with the spectral data previously reported for 4-amino-1-methylpyridazin-6-one[26] and clearly

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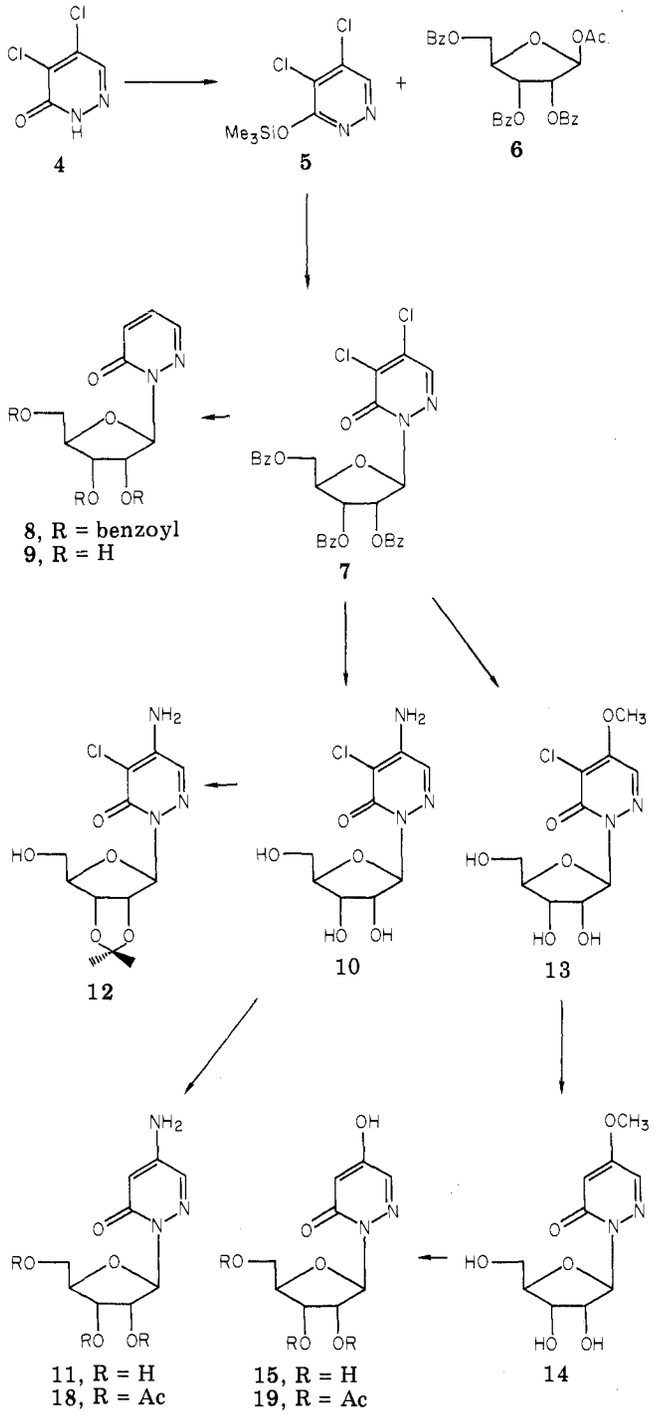
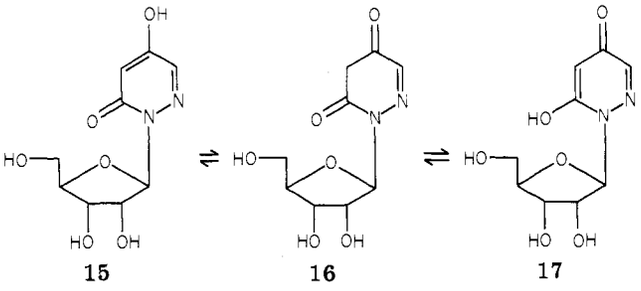
Scheme I



different from the spectral data reported[27] for the O-alkylated compound 5-amino-3-methoxypyridazine. Ultraviolet spectral data are summarized in Table II. This observation unequivocally establishes the site of ribosylation for **11** and the other nucleosides in this study which were derived from **7** as N-1. Furthermore, the coupling constant of the aromatic protons in **11** is characteristic of meta for the two protons ($J_{3,5}$ = 2.5 Hz).[24] This supports the contention that initial displacement of the 4-chloro (and not the 5-chloro) substituent had taken place.

Treatment of **10** with a catalytic amount of perchloric acid in dry acetone yielded the isopropylidene derivative 4-amino-5-chloro-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)pyridazin-6-one (**12**). The ^{1}H NMR spectrum

Scheme II



of **12** in dimethyl-d_6 sulfoxide exhibited a doublet ($J_{1',2'}$ = 1 Hz) at δ 6.36 for the anomeric proton and two singlets for the methyl groups of the acetonide moiety at δ 1.33 and 1.52 (Δδ = 0.19 ppm). The small coupling constant for the anomeric proton[28,29] and the Δδ value[30] for the two methyl signals of the acetonide moiety establish unequivocally a β-anomeric configuration for **12** and, therefore, a β-anomeric configuration for all of the nucleosides derived from **7**.

Reacting **7** with methanolic sodium methoxide at room temperature resulted in a displacement of the 4-chloro group and a concomitant removal of the benzoyl blocking groups to furnish a 93% yield of 5-chloro-4-methoxy-1-β-D-ribofuranosylpyridazin-6-one (**13**). Dehalogenation of **13** with hydrogen (40 psi) in the presence of 10% palladium on carbon catalyst in methanol gave a 91% yield of 4-methoxy-1-β-D-ribofuranosylpyridazin-6-one (**14**). A comparison of the ultraviolet spectra of N-1 methylated pyridazin-6-ones[31] structurally related to the nucleosides **13** and **14** lends further support for the site of ribosylation as N-1.

Hydrolysis of the 4-methoxy moiety of **14** was accomplished with aqueous potassium hydroxide heated at reflux temperature to provide an 84% yield of the desired uridine analogue 4-hydroxy-1-β-D-ribofuranosylpyridazin-6-one (**15**, 6-aza-3-deazauridine). Assignment of **15** to the 4-hydroxypyridazin-6-one structure rather than that of the tautomeric isomers **16** or **17** (Scheme II) is based on the similarity between the ultraviolet spectrum at pH 1 of **15** and that of **14** and a dissimilarity to a pH 1 ultraviolet spectrum reported[32] for 6-methoxy-1-β-D-ribofuranosylpyridazin-4-one. This assignment was also confirmed by an inspection of their ^{1}H NMR spectra. The 4-hydroxypyridazin-6-one structure has recently been confirmed for **15** in the solid state by an X-ray crystallographic study.[33] A pK_a determination by the method of Albert[34] yielded a value of 4.7 for the pK_a of the phenolic hydroxyl group of **15**, indicating that addition of a nitrogen atom in the 6-position of 3-deazauridine, which reportedly[35] has a pK_a of 6.2, increases the acidity of the phenolic hydroxyl moiety. This relates to a pK_a of 9.17 for uridine. Thus, at physiological pH, **15** would be expected to predominately exist in an ionized state. It is also interesting to note that deuterium exchange of the peak corresponding to H-5

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Table II. Ultraviolet Spectral Data for Some Pyridazine Nucleosides and Aglycons

	λ_{max}, nm ($\epsilon \times 10^{-3}$)				λ_{max}, nm ($\epsilon \times 10^{-3}$)		
no.	MeOH	pH 1	pH 11	no.	MeOH	pH 1	pH 11
7	275 sh (7.10)			**22**	245 (12.9)	237 (15.7)	246 (10.7)
	282 (7.40)				253 sh (12.2)	295 (9.14)	273 sh (6.28)
	297 (6.00)				294 sh (4.40)		365 (2.89)
8	275 (5.73)				360 (4.21)		
	281.5 (5.73)			**23**	242 (22.3)	243 (22.0)	243 (22.3)
9	293 (3.88)	286 (4.17)	286 (3.40)		300 sh (5.90)	295 sh (4.80)	295 sh (4.74)
10	277 (8.26)	276 (9.46)	277 (7.56)		326 (7.37)	328 (8.08)	328 (9.08)
	281 sh (8.08)	294 sh (7.86)	294 sh (6.36)	**24**	290 (6.72)	290 sh (6.42)	290 sh (5.01)
	299 sh (4.98)				305 sh (6.45)	307.5 (7.45)	310 (6.53)
11	277 (5.78)	275 (6.56)	275 (5.90)	**25**	240 (10.6)	240 (12.2)	240.5 (11.9)
	302 sh (3.10)	295 sh (4.46)	295 (3.93)		249 sh (10.2)	302 (8.16)	302 (7.97)
12	277 (5.86)	276 (6.19)	276 (6.25)		302 (7.74)	345 sh (4.86)	345 sh (4.86)
	291 sh (4.85)	290 sh (5.54)	290 (5.54)		340 (5.31)		
13	265 (4.45)	266 (6.70)	268 (2.72)	**26**	248 (9.41)	248 (7.80)	248 (7.05)
	296 (4.77)	292 (6.88)	292 (2.57)		303 (2.27)	305 (2.60)	307 (1.99)
14	246 (4.92)	247 (5.80)	248 (5.48)	**28**	282 (7.81)		
	278 (3.58)	270 sh (2.95)	270 sh (3.63)		300 sh (7.24)		
15	252 (4.10)	249 (4.63)	267 (6.20)	**29**	282 (6.13)		
	275 sh (3.75)	273 sh (3.49)	293 sh (3.82)		300 (6.26)		
18	273 (7.75)	273 (7.79)	273 (6.57)	**30**	278 (5.95)	276 (6.55)	276 (5.89)
	300 sh (4.76)	295 sh (5.54)	295 sh (3.84)		294 sh (4.72)	294 sh (5.53)	292 sh (4.82)
19	269 (5.76)	247 (6.22)	266 (8.27)	**31**	262 (4.13)	263 (4.99)	262 (4.46)
		273 sh (4.44)	292 sh (5.39)		294 (4.46)	289 (5.29)	289 (4.62)
20	299 (5.64)	296 (6.04)	295 (3.77)	**32**	275 (6.22)	274 (7.04)	274 (6.54)
	313 (5.61)	310 (6.92)	311 (6.45)		302 sh (3.29)	297 sh (4.09)	295 sh (4.06)
21	279 (7.40)	279 (8.12)	279 (7.43)	**33**	245 (5.30)	246 (5.91)	247 (5.40)
	300 sh (4.55)	297 sh (6.45)	297 sh (5.94)		277 (3.68)	270 sh (3.44)	270 sh (3.46)
				34	255 (5.08)	248 (5.88)	266 (8.80)
					270 (4.89)	273 sh (4.29)	292 sh (5.88)

in the ^{1}H NMR spectrum of **15** in dimethyl-d_6 sulfoxide occurred as shown by the disappearance of the C-5 proton signal and collapse of the C-3 proton signal to a singlet upon the addition of sodium deuterioxide in deuterium oxide, indicating that **15** is in equilibrium with its keto tautomer **16**.

Since acetylated derivatives of nucleoside antimetabolites have been prepared to improve the absorption properties, and thus the therapeutic efficacy, of the agents,[36] the tri-*O*-acetylribofuranosyl derivatives of nucleosides **11** and **15** were prepared for biological evaluation. Compound **11** was not easily acetylated using conventional methods but was acetylated using the acid-catalyzed procedure of Beranek and Drasar[37] to give a moderate yield of 4-amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-pyridazin-6-one (**18**). Compound **15**, on the other hand, could be acetylated under more standard conditions (acetic anhydride in pyridine) to give a good yield of 4-hydroxy-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyridazin-6-one (**19**).

In an effort to determine the generality of the substitution reactions used in the syntheses of compounds **11** and **15**, the dichloro nucleoside **7** was treated with a variety of other nucleophiles. When **7** was allowed to react with methylamine at 125 °C for 18 h, a single nucleoside product was formed. Spectral and analytical data indicated that displacement of one of the chloro substituents had taken place, in addition to a removal of the benzoyl blocking groups. Based on the previously noted reactivity of the 4,5-dichloropyridazin-6-one portion of compound **7**, the product was assigned the structure 5-chloro-4-(methylamino)-1-β-D-ribofuranosylpyridazin-6-one (**20**). Dehalogenation of **20** using hydrogen in the presence of palladium on carbon catalyst yielded 4-(methylamino)-1-β-D-ribofuranosylpyridazin-6-one (**21**). Inspection of the aromatic region of the ^{1}H NMR spectrum of **21** provided evidence that the initial nucleophilic substitution of the halogen atoms had occurred at the 4-position with two signals [δ 7.51 (d, H_3, $J_{3,5}$ = 2 Hz) and 5.34 (d, H_5, $J_{5,3}$ = 2 Hz). The reaction of nucleoside **7** with a stronger nucleophile, such as dimethylamine, at 120 °C for 19 h yielded two nucleoside products. One product was isolated in 17% yield after chromatography and gave spectral and analytical data which indicated that displacement of both the 4- and 5-chloro substituents had taken place, along with a removal of the benzoyl groups. This nucleoside was assigned the structure 4,5-bis(dimethylamino)-1-β-D-ribofuranosylpyridazin-6-one (**22**). Spectral and analytical data for the major product (isolated in 63% yield) indicated that displacement of only one chloro substituent had occurred, and it was assigned the structure 5-chloro-4-(dimethylamino)-1-β-D-ribofuranosylpyridazin-6-one (**23**). This assignment was based on the ^{1}H NMR spectrum obtained for the dehalogenated product 4-(dimethylamino)-1-β-D-ribofuranosylpyridazin-6-one (**24**, $J_{3,5}$ = 3 Hz), which was obtained from **23** by treatment with hydrogen in the presence of palladium on carbon.

Treatment of nucleoside **7** with 1 equiv of alkyl mercaptide anions at low temperature (0–5 °C), followed by a removal of the benzoyl blocking groups with methanolic sodium methoxide, led to inseparable mixtures of products. This situation was apparently due to the indiscriminate attack by these nucleophiles at the 4-chloro, 5-chloro, or at both positions in the molecule. However, treatment of **7** with 2 equiv of the nucleophiles, followed by deblocking, gave good yields of bis(alkylthio) nucleosides. By this procedure, 4,5-bis(methylthio)-1-β-D-ribofuranosylpyridazin-6-one (**25**) and 4,5-bis(benzylthio)-1-β-D-ribofuranosylpyridazin-6-one (**26**) were synthesized in 86 and 64% yields, respectively (Scheme III).

Deoxyribonucleosides of pyridazine heterocycles have been studied to a limited extent,[38-42] but once again the

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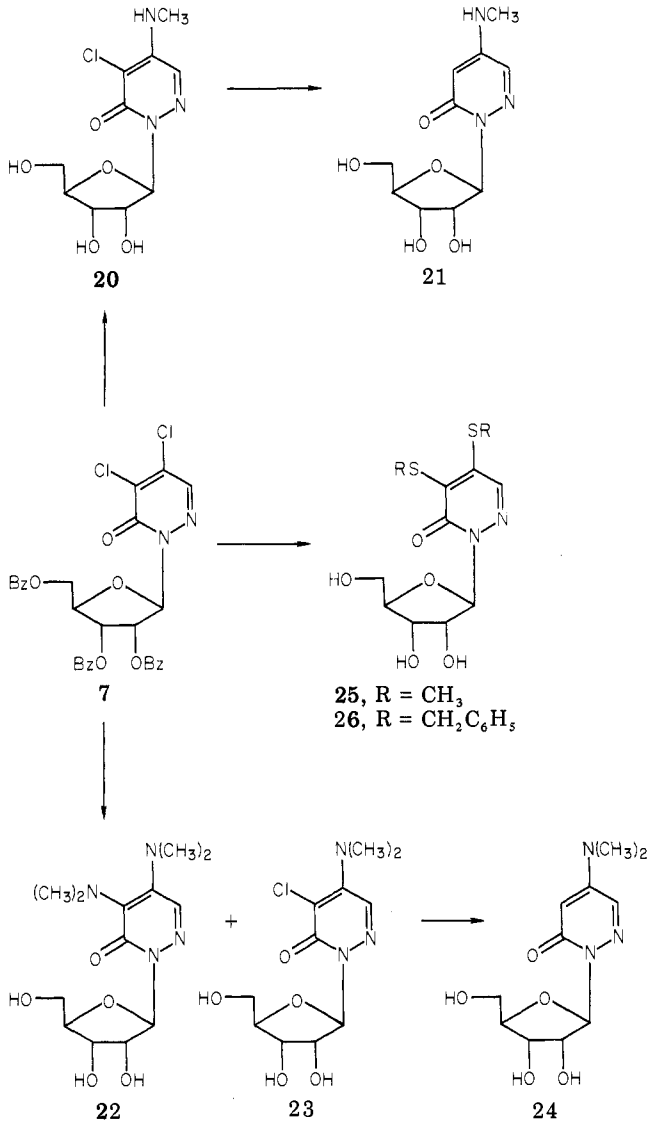
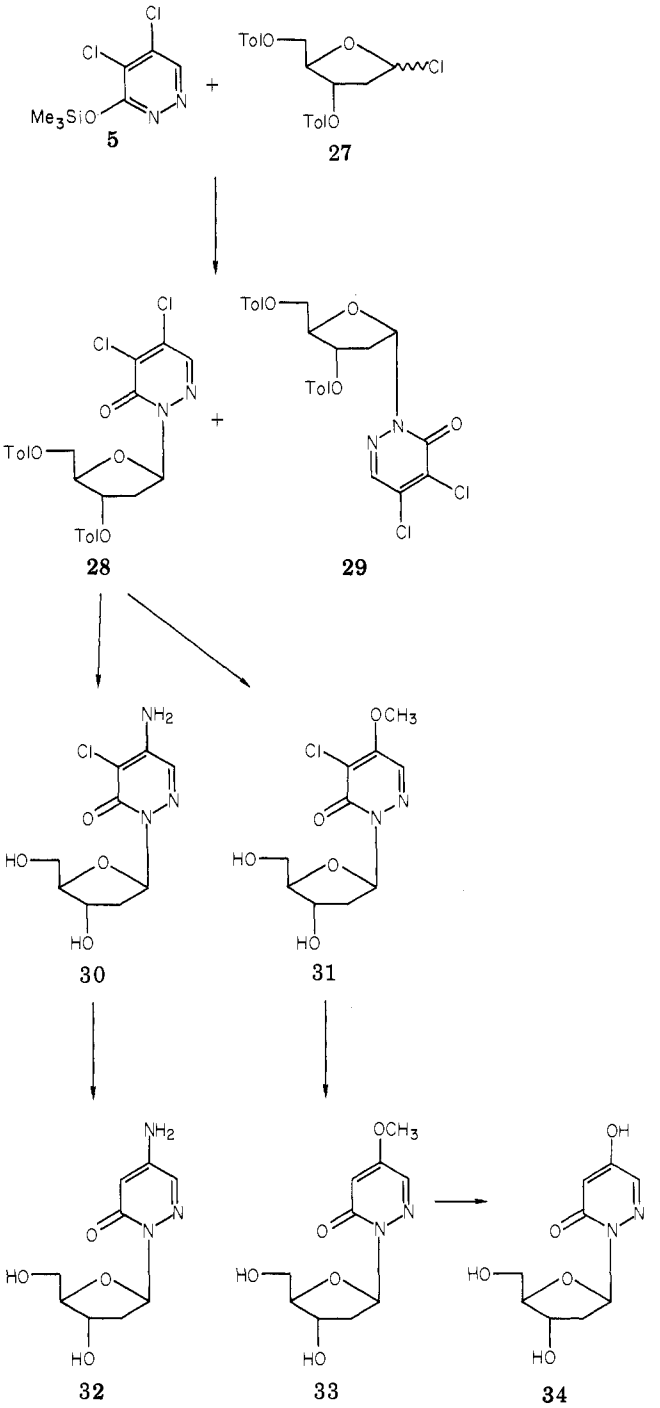
Scheme III



analogues of the naturally occurring compounds had not been reported. Condensation of the silyl derivative **5** with 3,5-di-*O*-*p*-toluoyl-D-*erythro*-pentofuranosyl chloride[43] (**27**) in dichloroethane at reflux in the presence of stannic chloride furnished two nucleoside products, **28** and **29**, in 24 and 30% yields, respectively. Identical ultraviolet and analytical data indicated that these products were an anomeric pair of N-1 glycosides of the starting heterocycle. Comparing the proton nuclear magnetic resonance spectra[44] of **28** and **29** permitted an assignment of their anomeric configurations. The ^{1}H NMR signal for the anomeric proton for the major product (**28**) appeared as a pseudotriplet [δ 6.93 ($J_{1',2'}$ = 6 Hz)], indicative[44] of the β configuration, while the analogous signal for compound **29** appeared as a doublet of doublet [δ 6.67 ($J_{1',2'}$ = 7 and

Scheme IV[a]



[a] Tol = tolouyl.

3 Hz), indicating that **29** possessed the α-anomeric configuration. In addition, compound **29** also exhibited a 0.32-ppm downfield chemical shift for $H_{4'}$ relative to the same proton in compound **28**. Previous studies have shown such a shift to be characteristic of the α anomers of pyridazine deoxyribosides.[41] Therefore, compounds **28** and **29** were assigned the structures 4,5-dichloro-1-(3,5-di-*O*-*p*-toluoyl-2-deoxy-β- and -α-D-*erythro*-pentofuranosyl)-pyridazin-6-one, respectively. The β anomer, **28**, was selected for further synthetic studies.

Transformations of **28** were performed essentially in an identical manner with those performed on the corresponding ribonucleosides; however, some added precautions were necessary because of the increased acid lability of the glycosyl bond of the resulting deoxynucleosides. Treatment of **28** with liquid ammonia at 110 °C effected

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a simultaneous removal of the toluoyl blocking groups and displacement of the 4-chloro substituent to yield 4-amino-5-chloro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-pyridazin-6-one (**30**) in 40% yield (Scheme IV). Dehalogenation of **35** with hydrogen (40 psi) in the presence of 10% palladium on carbon catalyst gave 4-amino-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyridazin-6-one (**32**, 6-aza-3-deaza-2′-deoxycytidine).

Treatment of **28** with methanolic sodium methoxide yielded 5-chloro-4-methoxy-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyridazin-6-one (**31**) in 79% yield. Dehalogenation of **31** with hydrogen (40 psi) and 10% palladium on carbon catalyst gave 4-methoxy-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyridazin-6-one (**33**). Alkaline hydrolysis of the 4-methoxy moiety of **33** yielded 4-hydroxy-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-pyridazin-6-one (**34**, 6-aza-3-deaza-2′-deoxyuridine). A comparison of the ultraviolet spectral data for compounds **30** through **34** with the spectra for the corresponding ribosides confirmed the site of 2′-deoxyribosylation of the heterocycle **4** as being at N-1.

Biology. All new compounds were tested for growth inhibition of L1210 mouse leukemic cells. Any compound which was found to significantly inhibit cell growth in culture at a concentration of 10^{-4} M was studied further to determine the concentration required to produce 50% inhibition, the ID_{50}. The cytotoxic effect was then investigated by treating cells for various time periods with a concentration of the test compound that would cause total growth inhibition if present continuously. The viability of the treated cells was then evaluated by colony formation and/or growth curve extrapolation. Treatments after which the cells resume growth are considered cytostatic, while those after which no cells are capable of proliferation are considered cytocidal. In this respect 6-aza-3-deazauridine (**15**) was found to inhibit the growth of L1210 cells with an ID_{50} of about 7×10^{-5} M.[45] Interestingly, preliminary studies[45] suggest that 6-aza-3-deazauridine (**15**) may inhibit cytidine triphosphate synthetase rather than orotidylate decarboxylation. The antitumor activity in vivo for **15** was marginal with ILS values of 32% at doses of 200 and 400 mg/kg for L1210-bearing mice. Since 3-deazauridine was found[46] to be significantly more active against a subline of L1210 resistant to arabinosylcytosine (L1210/*ara*-C) than it was against the parent sensitive line (L1210/0), the nucleoside **15** was also tested against this *ara*-C resistant subline. No significant increase in the ILS in the L1210/*ara*-C subline, however, was observed over the test in the parent line. All other compounds reported herein failed to inhibit in vitro L1210 growth at 10^{-4} M concentrations or exhibit an ILS of greater than 25% at 400 mg/kg and were considered inactive.

This investigation has provided the 6-aza-3-deaza derivatives of cytidine, uridine, 2′-deoxycytidine, and 2′-deoxyuridine. Additional chemical transformations of this novel group of pyrimidine nucleoside analogues, along with an evaluation of their biochemical properties, are currently under investigation in our laboratory.

Experimental Section

Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained with JEOL C60H, Varian A56/60, and Varian EM-390 spectrometers (solution in dimethyl-d_6 sulfoxide or chloroform-*d*) with chemical-shift values reported in δ units (parts per million) relative to an internal standard (sodium 2,2-dimethyl-2-silapentane-5-sulfonate or tetramethylsilane). Ultraviolet spectra were recorded on a Beckman Acta CIII spectrophotometer. Melting points were determined with a Thomas-Hoover capillary apparatus and are uncorrected. The optical rotations were measured with a Perkin-Elmer Model 141 polarimeter. Preparative thin-layer chromatography was performed on glass plates coated with silica gel (1.50 mm, SilicAR 7GF, Mallinckrodt). Compounds of interest were detected by either ultraviolet lamp (Mineralight, 254 nm) or treatment with sulfuric acid followed by charring. Open-bed column chromatography was carried out on SilicAR CC7 (Mallinckrodt) using gravity flow. The columns were packed as slurries with the elution solvent. All solvent proportions are given by volume. Evaporations were performed in a rotary evaporator under reduced pressure (water aspirator) or in vacuo at 40 °C unless otherwise stated. All compounds were dried in vacuo at 80 °C for 10 to 15 h before submission for elemental analysis. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ, and Heterocyclic Chemical Corp., Harrisonville, MO. The presence of water of crystallization in the elemental analyses was verified by ^{1}H NMR.

4,5-Dichloro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyridazin-6-one (7). 4,5-Dichloropyridazin-6-one[20] (**4**; 100 g, 0.61 mol) was silylated in 250 mL of hexamethyldisilazane by heating under reflux for 1.5 h in the presence of a catalytic amount of ammonium sulfate. The excess hexamethyldisilazane was removed by distillation under reduced pressure, and the resulting syrup was used without further purification. 1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**6**; 275 g, 0.54 mol) dissolved in 900 mL of dry dichloroethane was added in one portion to the above silyl derivative, and the mixture was stirred in an ice/water bath for 15 min. Stannic chloride (140 mL, 1.18 mol) was then added, and the reaction mixture was brought to immediate reflux. After 45 min, heating was discontinued and the reaction mixture was cooled to 10 °C in an ice/water bath. The reaction mixture was transferred to a 2-L beaker, and 300 mL of ethanol was added with stirring. Sodium bicarbonate (400 g, 4.76 mol) was added with stirring over a 4-h period, and the resulting gelatinous mass was allowed to air-dry. The resulting solid was ground to a powder and extracted in a Soxhlet extractor with hot chloroform. The chloroform extracts (700 mL) were added dropwise to 1200 mL of boiling methanol to effect precipitation of the product. After the solution was cooled to room temperature, the solid was collected by filtration to yield 270 g (0.44 mol, 82%) of white needles, mp 160–162 °C. Concentration of the filtrate to a volume of 300 mL and cooling to room temperature afforded an additional 18 g of product: total yield 288 g (0.47 mol, 88%); mp 163–164. Recrystallization of a small sample from methanol yielded an analytical sample, mp 166–167 °C; $[\alpha]^{27}_D$ −55.5° (*c* 1, chloroform). Anal. ($C_{30}H_{22}Cl_2N_2O_8$) C, H, N.

1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)pyridazin-6-one (8). Nucleoside **7** (1 g, 1.64 mmol) was dissolved in a mixture of 200 mL of methanol and 50 mL of tetrahydrofuran, the solution was cooled to 5 °C and then purged with nitrogen, 10% palladium on carbon catalyst (500 mg) was added, and the reaction mixture was treated with hydrogen gas at atmospheric pressure and room temperature for 6 h. The reaction mixtures from two such runs were combined, brought to a vigorous boil, and filtered through a Celite pad. The Celite pad was then washed with 400 mL of boiling methanol and the combined filtrates were concentrated under reduced pressure (50 °C) to ≃25 mL to give a white powder: yield 1.4 g (2.59 mmol, 79%); mp 95–100 °C. Recrystallization of a small sample from methanol yielded an analytical sample, mp 115–117 °C (lit.[17] mp 117 °C).

1-β-D-Ribofuranosylpyridazin-6-one (9). Nucleoside **8** (500 mg, 0.92 mmol) was dissolved in a mixture of 10 mL of tetrahydrofuran and 20 mL of methanol. Solid sodium methoxide (20 mg) was added, and the mixture was stirred for 1 h at room temperature. Amberlite IR-120 (H^+ form, 500 mg) was added, and the mixture was stirred for an additional 2 h at room temperature. The resin was separated by filtration and washed with 50 mL of boiling methanol. The combined filtrates were evaporated under reduced pressure at room temperature to yield an oil, which was crystallized from a mixture of ethyl acetate and

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methanol. The beige crystals were collected by filtration: yield 160 mg (70 mmol, 76%); mp 168–169 °C (lit.[24] mp 171.7–173.5 °C).

4-Amino-5-chloro-1-β-D-ribofuranosylpyridazin-6-one (10). Nucleoside **7** (30 g, 49 mmol) was suspended in 300 mL of liquid ammonia, and the mixture was heated at 110 °C in a sealed stainless-steel reaction vessel for 10 h. The excess ammonia was allowed to evaporate at room temperature. The remaining residue was triturated with chloroform (2 × 250 mL) to remove benzamide and any remaining partially blocked nucleosides. The powdery residue was crystallized from water to yield 9 g (32 mmol, 66%) of product, mp 238–240 °C. Recrystallization of a small sample from water yielded an analytical sample, mp 243–244 °C; $[\alpha]^{27}_D$ −132° (*c* 1, dimethylformamide). Anal. ($C_9H_{12}ClN_3O_5 \cdot 0.5H_2O$) C, H, N.

4-Amino-1-β-D-ribofuranosylpyridazin-6-one (11, 6-Aza-3-deazacytidine). Nucleoside **10** (15 g, 52 mmol) was suspended in 500 mL of water and 8 mL of 30% aqueous sodium hydroxide. After the mixture was purged with nitrogen, 2 g of 10% palladium on carbon catalyst was added, and the mixture was treated with hydrogen gas at 40 psi in a Parr hydrogenation apparatus for 16 h at room temperature. The mixture was filtered through a Celite pad, and the pad was washed with 300 mL of boiling water. The filtrates were combined and evaporated in vacuo to dryness at 50 °C. The residue was triturated with ethanol (5 × 200 mL), and the combined ethanol washings were evaporated under reduced pressure to dryness at room temperature to yield 7.9 g (31 mmol, 60%) of crude product, mp 217–220 °C. Recrystallization of a small sample from water yielded an analytical sample, mp 228–229 °C; $[\alpha]^{27}_D$ −134° (*c* 1, dimethylformamide). Anal. ($C_9H_{13}N_3O_5 \cdot 0.5H_2O$) C, H, N.

4-Amino-5-chloro-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)pyridazin-6-one (12). A mixture of dry acetone (42 mL) and acetone dimethyl acetal (1 mL) was cooled to 0 °C in an ice/water bath. Perchloric acid (1 drop), followed by 155 mg (0.54 mmol) of nucleoside **10**, was added to this mixture, and the solution was stirred at room temperature for 12 h. The solution was then neutralized with solid sodium bicarbonate and filtered by suction through a Celite pad. The filtrate was concentrated to dryness at room temperature in vacuo, and the residue was coevaporated with methanol. The resulting oil was crystallized from cyclohexane to yield a yellow solid (88 mg, 0.27 mmol, 50%), which did not give a distinct melting point. A portion of the solid was recrystallized from water to yield an analytical sample, mp 176–177 °C. Anal. ($C_{12}H_{16}ClN_3O_5 \cdot H_2O$) C, H, N.

5-Chloro-4-methoxy-1-β-D-ribofuranosylpyridazin-6-one (13). Nucleoside **7** (50 g, 82 mmol) was dissolved in tetrahydrofuran (300 mL), and methanol (300 mL) was added. Sodium methoxide (7 g, 140 mmol) was then added, and the solution was stirred for 24 h at room temperature. Amberlite IR-120 resin (H^+ form, 20 g) was added, and the solution was then stirred for an additional 24 h. The mixture was filtered, and the resin was washed with 200 mL of boiling methanol. The combined filtrates were evaporated under reduced pressure to yield an oil, which was crystallized from ethyl acetate (200 mL) by the addition of a small amount of methanol. Suction filtration gave a white powder: yield 19.9 g (68 mmol, 83%); mp 150–160 °C. Recrystallization from a small amount of the powder from ethyl acetate yielded an analytical sample: mp 161–163 °C; $[\alpha]^{27}_D$ −94° (*c* 1, methanol). Anal. ($C_{10}H_{13}ClN_2O_6$) C, H, N.

4-Methoxy-1-β-D-ribofuranosylpyridazin-6-one (14). Nucleoside **13** (20 g, 68 mmol) was dissolved in methanol (600 mL), and 15 mL of a 30% aqueous sodium hydroxide solution was then added. The solution was purged with nitrogen for 0.5 h and cooled to 0 °C. Palladium on carbon catalyst (10%; 2 g) was added, and the reaction mixture was then treated at room temperature with hydrogen gas at 40 psi in a Parr hydrogenation apparatus for 12 h. The reaction mixture was heated to boiling and filtered through a Celite pad. The pad was washed with 500 mL of boiling methanol, and the combined filtrates were evaporated to dryness under reduced pressure. The residue was triturated with 600 mL of ethanol at room temperature and filtered by suction, and the ethanol filtrate was evaporated under reduced pressure at room temperature. The resulting residue was crystallized from ethyl acetate with the addition of a small amount of methanol to yield 16.6 g (60 mmol, 91%) of **14**, mp 135–140 °C. Recrystallization of a small sample from an ethyl acetate/methanol mixture yielded an analytical sample: mp 143–144 °C; $[\alpha]^{27}_D$ −132° (*c* 1, methanol). Anal. ($C_{10}H_{14}N_2O_6 \cdot 0.5H_2O$) C, H, N.

4-Hydroxy-1-β-D-ribofuranosylpyridazin-6-one (15, 6-Aza-3-deazauridine). Nucleoside **14** (10 g, 37 mmol) was added to 200 mL of distilled water containing 40 g of potassium hydroxide, and the solution was heated at reflux for 3 h. The reaction mixture was then cooled in an ice bath, and 57 mL of concentrated hydrochloric acid was added dropwise with constant stirring. During the addition, care was taken to maintain the temperature in the reaction flask below 10 °C. The resulting acidic solution was evaporated to dryness at 30 °C in vacuo, and the residue was triturated at room temperature with 500 mL of ethanol for 15 h. The mixture was filtered by suction, and the filtrate was evaporated under reduced pressure to yield an oil, which was crystallized from ethyl acetate to give a light yellow powder: yield 7.5 g (31 mmol, 84%); mp 155–160 °C. Recrystallization of a small sample from an ethyl acetate/ethanol mixture yielded an analytical sample: mp 186–187 °C; ^1H NMR (Me_2SO-d_6) δ 7.92 (d, 1 H, H_3, $J_{3,5}$ = 2.5 Hz), 6.22 (d, 1 H, $H_{1'}$, $J_{1',2'}$ = 3.5 Hz), 6.10 (d, 1 H, H_5, $J_{5,3}$ = 2.5 Hz). Deuterium exchange by addition of deuterium oxide and sodium deuterioxide caused the peak at δ 6.10 (H_5) to disappear with a corresponding collapse of the peak at δ 7.92 (H_3) to a singlet: $[\alpha]^{27}_D$ −133° (*c* 1, methanol). Anal. ($C_9H_{12}N_2O_6 \cdot 0.5H_2O$) C, H, N.

4-Amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-pyridazin-6-one (18). Nucleoside **11** (1 g, 4.12 mmol) was dissolved in 6 mL of acetic acid. Acetyl chloride (2 mL) was added, and the mixture was stirred for 10 min at room temperature. Chloroform (15 mL) was added, and the mixture was stirred for an additional 12 h. The reaction mixture was absorbed onto 1 g of silica gel and applied to an open-bed silica gel column (5 × 20 cm). The column was eluted with methanol/chloroform (1:19, v/v), and the fractions containing nucleoside material (as determined by TLC) were collected and evaporated under reduced pressure at about 30 °C to provide an oily residue. Crystallization from a mixture of isopropyl alcohol and cyclohexane gave beige crystals: yield 450 mg (1.22 mmol, 30%); mp 143–144 °C. Anal. ($C_{15}H_{19}N_3O_8$) C, H, N.

4-Hydroxy-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-pyridazin-6-one (19). Nucleoside **15** (1 g, 4.10 mmol) was dissolved in 15 mL of dry pyridine. Acetic anhydride (2 mL, 21 mmol) was added, and the mixture was stirred 4 h at room temperature. Methanol (5 mL) was then added, and the mixture was stirred for an additional 10 min. The solution was poured into a mixture of chloroform (100 mL) and water (50 mL) and shaken, and the layers were separated. The chloroform layer was washed with cold 1 N hydrochloric acid (5 × 50 mL) and dried over magnesium sulfate. After filtration, the filtrate was coevaporated with 1 g of silica gel, and the residue was applied to an open-bed silica gel column (4 × 10 cm). The column was eluted with methanol/chloroform (1:9, v/v), and the eluant containing the product (as determined by TLC) was evaporated under reduced pressure to give a homogeneous foam: yield 850 mg (2.24 mmol, 55%). Anal. ($C_{15}H_{18}N_2O_9 \cdot 0.5H_2O$) C, H, N.

5-Chloro-4-(methylamino)-1-β-D-ribofuranosylpyridazin-6-one (20). Nucleoside **7** (2 g, 3.28 mmol) was suspended in methylamine (20 mL) and heated in a steel reaction vessel for 18 h at 125 °C. The excess methylamine was allowed to evaporate at room temperature. The remaining solid mixture was triturated with chloroform (100 mL) to remove *N*-methylbenzamide and any remaining partially blocked nucleosides. The remaining residue was crystallized from water to give white needles: yield 680 mg (2.33 mmol, 71%); mp 225–226 °C. Anal. ($C_{10}H_{14}ClN_3O_5$) C, H, N.

4-(Methylamino)-1-β-D-ribofuranosylpyridazin-6-one (21). Nucleoside **20** (25 mg, 0.86 mmol) was dissolved in 50 mL of water, and the solution was cooled to 5 °C and then purged with nitrogen. Palladium on carbon catalyst (10%; 100 mg) suspended in water (10 mL) was added, and the mixture was treated with hydrogen gas at 40 psi and toom temperature for 22 h in a Parr hydrogenation apparatus. The mixture was filtered through a Celite pad, and the pad was washed with boiling water (100 mL). The combined filtrates were concentrated in vacuo to a volume of 10 mL. This solution was applied to a preparative thick-layer chromatographic plate (20 × 40 cm), and the plate was developed

with methanol/chloroform (3:17, v/v). The UV-absorbing band (R_f 0.2) was removed, and the silica gel was extracted with boiling methanol (200 mL) and filtered by suction. The filtrate was evaporated to dryness under reduced pressure, and the resulting solid was crystallized from methanol to give crude **21**: yield 90 mg (0.35 mmol, 41%); mp 222–225 °C. Recrystallization of a small sample from methanol yielded beige crystals, mp 229–230 °C. Anal. ($C_{10}H_{15}N_3O_5$) C, H, N.

4,5-Bis(dimethylamino)-1-β-D-ribofuranosylpyridazin-6-one (22) and 5-Chloro-4-(dimethylamino)-1-β-D-ribofuranosylpyridazin-6-one (23). Nucleoside **7** (2 g, 3.28 mmol) was suspended in dimethylamine (20 mL) and sealed in a stainless-steel reaction vessel. The mixture was heated at 120 °C for 19 h and then cooled to 0 °C. Excess dimethylamine was allowed to evaporate at room temperature. The residue from the reaction was dissolved in chloroform (20 mL), and the solution was applied to an open-bed silica gel column (5 × 15 cm). The column was eluted with chloroform (1 L) and then with methanol/chloroform (1:19, v/v, 700 mL). Elution was continued, and 20-mL fractions were collected. Nucleoside material was detected by TLC in fractions 9 through 27 and fractions 34 through 55. Fractions 9 through 27 were combined and evaporated to dryness under reduced pressure. The residue was crystallized from ethyl acetate (15 mL) to give **22** as white needles: yield 180 mg (0.57 mmol, 17%); mp 137–138 °C. Anal. ($C_{13}H_{22}N_4O_5$) C, H, N.

Fractions 34 through 55 were combined and evaporated to dryness under reduced pressure, and the residue was crystallized from ethyl acetate (15 mL) to yield **23** as a white powder: yield 630 mg (2.06 mmol, 63%); mp 170–171 °C. Anal. ($C_{11}H_{16}ClN_3O_5$) C, H, N.

4-(Dimethylamino)-1-β-D-ribofuranosylpyridazin-6-one (24). Nucleoside **23** (250 mg, 0.82 mmol) was dissolved in a mixture of methanol (50 mL) and water (10 mL). The mixture was cooled to 5 °C and nitrogen was then passed through the solution for 15 min. Palladium on carbon catalyst (10%; 100 mg as a slurry in 10 mL of water) was added, and the mixture was treated with hydrogen gas (40 psi) in a Parr hydrogenation apparatus for 22 h at room temperature. The reaction mixture was heated to boiling and filtered through a Celite pad, and the pad was washed with boiling methanol (100 mL). The combined filtrates were concentrated under reduced pressure to a volume of 10 mL. This solution was applied to a preparative thick-layer chromatographic plate (20 × 40 cm), and the plate was developed with methanol/chloroform (3:17, v/v). The band having R_f 0.3 was removed, and the silica gel was washed with boiling methanol (200 mL). Following suction filtration, the methanol solution was evaporated under reduced pressure, and the resulting solid was crystallized from methanol to give white crystals: yield 213 mg (0.79 mmol, 96%); mp 170–171 °C. Anal. ($C_{11}H_{17}N_3O_5$) C, H, N.

4,5-Bis(methylthio)-1-β-D-ribofuranosylpyridazin-6-one (25). Nucleoside **7** (2 g, 3.28 mmol) was dissolved in tetrahydrofuran (50 mL), and the solution was cooled to 10 °C. A 0.09 M methanolic solution (80 mL, 7.2 mmol) of sodium methyl mercaptide was added, and the reaction mixture was stirred at 10 °C for 0.5 h. Sodium methoxide (1 g) dissolved in methanol (100 mL) was added, and the mixture was stirred for 12 h at room temperature. Amberlite IR-120 resin (H^+ forms, 2 g) was added, the mixture was stirred for 3 h and filtered, and the resin was washed with methanol (100 mL). The reaction mixtures from two such runs were combined and evaporated under reduced pressure at room temperature to yield an oil. This oil was crystallized from ethyl acetate to give white needles: yield 1.8 g (5.63 mmol, 86%); mp 140–142 °C. Anal. ($C_{11}H_{16}N_2O_5S_2$) C, H, N.

4,5-Bis(benzylthio)-1-β-D-ribofuranosylpyridazin-6-one (26). Benzyl mercaptan (0.5 mL, 4.27 mmol) was dissolved in tetrahydrofuran (50 mL), and sodium metal (75 mg, 3.28 mmol) was added. After the mixture was stirred for 2 h, the resulting suspension was used without further purification. Nucleoside **7** (2 g, 3.28 mmol) was added to the suspension of sodium benzyl mercaptide, and the mixture was stirred for 3 h at room temperature. An additional 3.28 mmol of sodium benzyl mercaptide (prepared as above) was added, and the mixture was stirred for an additional 1 h. Two such reaction mixtures were combined and diluted with methanol (200 mL). Sodium methoxide (2 g) was added, and the mixture was then stirred for 12 h at room temperature. Amberlite IR-120 resin (H^+ form, 2 g) was added, and the mixture was stirred for 3 h. The mixture was filtered and the resin was washed with methanol (100 mL). The combined filtrates were concentrated to an oily residue, which was applied to an open-bed silica gel column (5 × 12.5 cm). The column was washed with chloroform (1.5 L) and methanol/chloroform (1:49, v/v, 500 mL) to remove methyl benzoate and any remaining blocked nucleosides. The main product, as detected by TLC, was then eluted from the column with 500 mL of methanol/chloroform (1:1, v/v). The eluant containing product was then concentrated to dryness under reduced pressure to yield an oil, which crystallized from methanol/chloroform (1:1, 100 mL) to furnish yellow needles: yield 2.2 g (4.65 mmol, 64%); mp 79–81 °C. Anal. ($C_{23}H_{24}N_2O_5S_2$) C, H, N.

4,5-Dichloro-1-(3,5-di-*O*-*p*-toluoyl-2-deoxy-β- and -α-D-*erythro*-pentofuranosyl)pyridazin-6-one (28 and 29). 4,5-Dichloropyridazin-6-one[20] (**4**; 8 g, 48.5 mmol) was heated at reflux for 3 h in hexamethyldisilazane (100 mL) containing a catalytic amount of $(NH_4)_2SO_4$, and the mixture was allowed to cool to room temperature. The excess hexamethyldisilazane was removed by distillation (aspirator pressure, 40 °C) and the residual silyl derivative was used without further purification. The silyl derivative was dissolved in dichloroethane (100 mL), and the solution was cooled to 5 °C. A solution of halogenose **27**[43] (20 g, 51.6 mmol) in dichloroethane (100 mL) was cooled to 5 °C and added to the solution of the silyl derivative. Stannic chloride (3 mL, 25.3 mmol) was added, and the reaction mixture was stirred for 5 min at 5 °C. The reaction mixture was brought to reflux temperature, heated under reflux for 15 min, and then immediately cooled to 5 °C. Ethanol (50 mL) was added to the mixture and then sodium bicarbonate (6 g, 71 mmol) was added over a 3-h period. The resulting gelatinous mass was allowed to air-dry for 20 h to yield a solid residue. The residue was pulverized and then extracted for 8 h in a Soxhlet extractor using chloroform as the solvent. The chloroform extract was concentrated under reduced pressure to a syrup, which was then dissolved in hexane/chloroform (3:7, v/v, 75 mL). The solution was applied to an open-bed silica gel column (4.5 × 45 cm), and the products were then eluted from the column using the same solvent system. The first 625 mL of eluant contained no nucleoside material. Fractions (200 mL) were then collected with the following results: Fractions 1 through 7 were combined and evaporated to dryness under reduced pressure to yield a solid. Boiling ethanol (50 mL) was added to the solid, and this solution was allowed to cool to room temperature (4 h) and filtered to give **28** as white needles: yield 6 g (11.6 mmol, 24%); mp 150–151 °C; $[\alpha]^{25}_D$ −133° (*c* 1, chloroform). Anal. ($C_{25}H_{22}Cl_2N_2O_6$) C, H, N.

Fractions 10 through 14 were combined and evaporated to dryness under reduced pressure to give **29** as a homogeneous oil: yield 5 g (9.66 mmol, 20%). Upon standing, the oil deposited white needles, which were collected and washed with ethanol: mp 91–93 °C; $[\alpha]^{25}_D$ +23° (*c* 1, chloroform). Anal. ($C_{25}H_{22}Cl_2N_2O_6$) C, H, N.

4-Amino-5-chloro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyridazin-6-one (30). Nucleoside **28** (1 g, 1.93 mmol) was suspended in liquid ammonia (20 mL) and sealed in a stainless-steel reaction vessel. The reaction was heated at 110 °C for 17.5 h and then allowed to cool to room temperature. Excess ammonia was allowed to evaporate at room temperature, and the remaining residue was washed with chloroform (7 × 25 mL). The remaining solid was dissolved in methanol (25 mL) and coevaporated with 1 g of silica gel. The silica gel was applied to the top of an open-bed silica gel column (5 × 6 cm), and the column was eluted with methanol/chloroform (3:17, v/v, 190 mL). The eluant, as determined by TLC, containing product was concentrated, and the resulting solid was crystallized from cyclohexane/ethyl acetate (8:2) to give a white powder: yield 200 mg (0.76 mmol, 40%); mp 181–182 °C. Anal. ($C_9H_{12}ClN_3O_4$) C, H, N.

4-Amino-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-pyridazin-6-one (32, 6-Aza-3-deaza-2′-deoxycytidine). Nucleoside **30** (100 mg, 0.38 mmol) was dissolved in distilled water (50 mL) which contained 0.4 mL of 0.1 N sodium hydroxide. The solution was cooled to 5 °C and thoroughly purged with nitrogen. Palladium on carbon catalyst (10%; 100 mg) was added, and the

reaction mixture was then treated with hydrogen gas at 40 psi in a Parr hydrogenation apparatus for 16 h at room temperature. The mixture was filtered through a Celite pad, and the pad was then washed with boiling water (50 mL). The filtrate was concentrated in vacuo to a volume of 5 mL, applied to a preparative thick-layer chromatographic plate, and then developed with methanol/chloroform (1:9, v/v). The main product (R_f 0.2) was collected by extraction of the silica gel with boiling methanol (100 mL). Evaporation of the methanol under reduced pressure gave **32** as a homogeneous oil: yield 60 mg (0.26 mmol, 70%). Crystallization of a small sample from methanol yielded an analytical sample, mp 145-146 °C. Anal. ($C_9H_{13}N_3O_4$) C, H, N.

5-Chloro-4-methoxy-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyridazin-6-one (31). Nucleoside **28** (2 g, 3.87 mmol) was dissolved in a mixture of methanol (50 mL) and tetrahydrofuran (35 mL). Sodium methoxide (300 mg, 5.6 mmol) was added, and the reaction mixture was stirred for 24 h at room temperature. Amberlite IR-120 resin (2 g, H^+ form) was added, and the mixture was stirred for an additional 3 h. The mixture was filtered and the resin was washed with boiling methanol (100 mL). The combined methanol washings were evaporated under reduced pressure to an oily mixture. Ethyl acetate (100 mL) and methanol (20 mL) were added to the mixture, and the solution was stored at 5 °C. After 2 h, the precipitated salts were removed by filtration, and the filtrate was concentrated to an oil. This oil was crystallized from chloroform (25 mL) to give **31** as a white powder: yield 850 mg (3.07 mmol, 79%); mp 154-156 °C. Anal. ($C_{10}H_{13}ClN_2O_5$) C, H, N.

4-Methoxy-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-pyridazin-6-one (33). Nucleoside **31** (500 mg, 1.18 mmol) was dissolved in methanol (100 mL) which contained 1.5 mL of 1 N sodium hydroxide. The reaction mixture was cooled to 5 °C and thoroughly purged with nitrogen. Palladium on carbon catalyst (10%; 200 mg) was added, and the mixture was then treated with hydrogen gas (40 psi) in a Parr hydrogenation apparatus for 13 h at room temperature. The reaction mixture was boiled and filtered through a Celite pad, and the pad was washed with boiling methanol (100 mL). The combined filtrates were concentrated under reduced pressure to an oil, which was triturated with ethanol (50 mL) at room temperature for 20 h. After filtration, the ethanol filtrate was coevaporated with 2 g of silica gel. This silica gel was then applied to an open-bed column (4.5 × 10 cm), and elution was performed with methanol/chloroform (1:19, v/v). Collecting 15-mL fractions, the product was eluted in fractions 30 through 50. These fractions were combined and then evaporated under reduced pressure to an oil, which was crystallized from cyclohexane to yield **33** as beige crystals: yield 260 mg (1.07 mmol, 59%); mp 122-124 °C. Anal. ($C_{10}H_{14}N_2O_5$) C, H, N.

4-Hydroxy-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-pyridazin-6-one (34, 6-Aza-3-deaza-2′-deoxyuridine). Potassium hydroxide (2 g) was dissolved in distilled water (10 mL). Nucleoside **33** (400 mg, 1.65 mmol) was added, and the mixture was heated at reflux for 1 h. The reaction mixture was cooled to 0 °C and the pH of the solution was adjusted to pH 1 with concentrated hydrochloric acid (cooling was maintained throughout the addition). The mixture was evaporated to dryness in vacuo at 30 °C, and the resulting residue was triturated with ethanol (30 mL) at room temperature for 3 h. The precipitated salts were separated by filtration, and the filtrate was concentrated under reduced pressure to an oil, which was crystallized from ethyl acetate to give **34** as a beige powder: yield 370 mg (1.56 mmol, 95%); mp 161-162 °C. Preparative thick-layer chromatography yielded an analytical sample, mp 173-174 °C. Anal. ($C_9H_{12}N_2O_5 \cdot 0.5H_2O$) C, H, N.

Antitumor Studies. The in vitro cytotoxicity against L1210 was evaluated as described previously.[47] L1210 cells were grown in static suspension culture using Fischers medium for leukemic cells of mice, and the growth rate over a 3-day period was determined in the presence of various concentrations of the test compound. The ID_{50} was determined as the concentration required to reduce the growth rate to 50% of the control.

The in vivo antitumor data was furnished by the Division of Cancer Treatment using standard National Cancer Institute Protocols for evaluation of compounds against the mouse leukemias L1210.[48]

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Synthesis of Some Potential Antihypertensive Phthalazinyl- and Quinoxalinylguanidines

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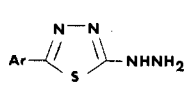
A series of phthalazinyl- and quinoxalinylguanidines has been synthesized and evaluated for potential antihypertensive activity. Unsubstituted guanidines were prepared by treating the appropriate intermediate chloro compounds with guanidine free base. Substituted guanidines were prepared by treating the cyanamides **9** and **16** with the appropriate amine; with hydrazines, cyanamide **9** gave the triazoles **14** and **15**. Moderate falls in blood pressure were observed with compounds **10**, **11**, **14**, and **15**. The triazole **15** caused a 25% fall in heart rate. Some of the compounds (**10**, **11**, **13**, and **18**) displayed weak α-adrenoceptor antagonist properties in vitro, and this activity was confirmed in the pithed rat (in vivo).

2-Aryl-5-hydrazino-1,3,4-thiadiazoles, exemplified by the general structure **1**[1] were designed as analogues of a compound (**2**)[2] known to possess vasodilator activity, the ethylene linkage in **2** being replaced by the bioisosteric "S" atom to give the thiadiazole ring in **1**. The hydrazines **1** were shown to be potent vasodilators,[1] and subsequently it was found that the corresponding thiadiazolylguanidines and aminoimidazolines **3**[3] possessed the same profile of activity, albeit of lower potency.



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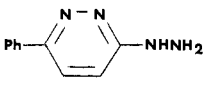

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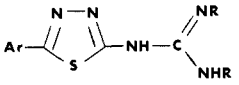

3 R = H or $-(CH_2)_2-$

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