280 mg (9%) of white needles: mp 200–201°; $\lambda_{max}^{0.1N\ HCl}$ (pH 8–13), 305 mμ; (pH 1), 283 mμ; $pK_a = 7.5$.

Anal. Calcd for $C_{18}H_{16}BrN_3O_4$: C, 48.8; H, 3.15; N, 13.1. Found: C, 49.0; H, 3.38; N, 13.0.

6-Benzyl-5-bromo-3-(*m*-nitrobenzyl)uracil (XXX).—A mixture of 2.18 g (7.77 mmoles) of 6-benzyl-5-bromouracil (XVII),[6] 25 ml of DMSO, 1.00 g (5.90 mmoles) of *m*-nitrobenzyl chloride, and 0.81 g (5.90 mmoles) of anhydrous K_2CO_3 was heated in a steam bath for 4 hr, then poured into 50 ml of cold water. After about 18 hr at 5°, the mixture was filtered and the solids were washed with water. Preparative tlc as described earlier[6] gave a zone for XXX that was eluted to give 320 mg (9.9%) of colorless prisms: mp 199–200°; λ_{max} (EtOH), 268 mμ; (0.1*N* EtOH, pH 13), 260, 310 mμ (sh).

Anal. Calcd for $C_{18}H_{14}BrN_3O_4$: C, 51.9; H, 3.39; N, 10.1. Found: C, 51.8; H, 3.53; N, 10.5.

Approaches to the Synthesis of 1-Deazauridine and 2′-Deoxy-1-deazauridine[1]

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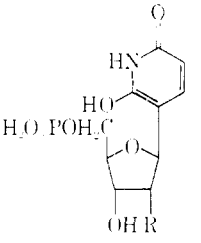
Received November 26, 1966

The synthesis of the title compounds as potential inhibitors of thymidylate synthetase was approached by modification of the procedure used in the synthesis of pseudouridine. Treatment of 3-bromo-2,6-dibenzyloxypyridine (**7b**) with *n*-butyllithium was followed by conversion to the 3-cadmium derivative **10b**. When the latter was treated with 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride followed by esterolysis, both 1,2-O-[3-(2,6-dibenzyloxypyridyl)]benzylidene-α-D-ribofuranose (**15**) and 3-(D-ribofuranosyl)-2,6-dibenzyloxypyridine (**14**) were obtained in 25 and 10% yields, respectively. The 2′-deoxy analog (**18**) was obtained by treatment of **10b** with 3,5-di-O-*p*-tolyl-2-deoxy-D-ribofuranosyl chloride and subsequent esterolysis. Hydrogenolysis of **14** and **18** gave the title compounds, both of which were unstable. In view of the latter the anomeric configuration and the cyclic nature of the sugar in **21** and **20** have not been determined. No significant inhibition of thymidylate synthetase or dihydrofolate reductase was noted in this series of compounds.

Strong inhibition of the thymidylate synthetase catalyzed conversion of deoxyuridine 5′-monophosphate to thymidine 5′-monophosphate has been reported for analogs of both the substrate and the product. The substrate analog, 5-fluoro-2′-deoxyuridine 5′-monophosphate (FUdRP), has a K_i of 5×10^{-8} *M* while the product analog, 5-trifluoromethyl-2′-deoxyuridine 5′-monophosphate (F_3dTMP), is reported to have a K_i of 4×10^{-8} *M*.[3] Baker[4] attributed the inhibition by FUdRP to enhanced electrostatic binding to the enzyme due to the increased acidity of the pyrimidine ring. In application of his proposed mechanism for inhibition of thymidylate synthetase 5-fluorouracil ($pK_a = 8.15$) and 5-trifluoromethyluracil ($pK_a = 7.35$), more acidic than their natural counterparts (uracil, $pK_a = 9.45$; thymine, $pK_a = 9.82$), as the 2′-deoxynucleotides could cause inhibition by increased binding affinity to the enzyme through the $N^3HC^4{=}O$ portion of the pyrimidine ring.[3]

Recently 5-trifluoromethyl-6-aza-2′-deoxyuridine and the 5′-monophosphate have been synthesized in an effort to further evaluate the effects of acidity on binding to this enzyme.[5] In contrast to the fluoro and trifluoromethyl compounds the fluorinated triazines, 5-trifluoromethyl-6-azauracil ($pK_a = 5.4$), its 2′-deoxynucleoside, as well as the 5′-monophosphate derivative, have been found to lack significant activity against thymidylate synthetase.

The acidity of 2,6-dihydroxypyridine (**8**, $pK_a = 4.2$, titration), which can be considered a deaza analog of uracil, prompted the synthesis of the respective sugar analogs, 1-deazauridine 5′-monophosphate (**1a**) and 2′-deoxy-1-deazauridine 5′-monophosphate (**1b**), to evaluate the effect of increased acidity on thymidylate synthetase inhibition.



1a, R = OH
b, R = H

In the search for new or enhanced biological activities the substitution of carbon for nitrogen has been reported for many classes of medicinal agents. Notable among the anticancer agents are the deaza analogs of the folic acid antimetabolites[6] and various purine analogs.[7] 3-Diazocitrazinic acid, a deaza analog of orotic acid, is reported to be a competitive antagonist of orotic acid.[8]

(1) (a) Taken in part from the dissertation presented by J. Zielinski to the Graduate School of the University of Kansas in partial fulfillment of the requirements for the degree of Doctor of Philosophy. (b) This work was generously supported by Grant CA-6536 from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

(2) The Kansas Division of the American Cancer Society is acknowledged for a partial summer fellowship.

(3) The chemistry and biology of these inhibitors have been reviewed recently: C. Heidelberger, *Progr. Nucleic Acid Res. Mol. Biol.*, **4**, 1 (1965); P. Reyes and C. Heidelberger, *Mol. Pharmacol.*, **1**, 14 (1965).

(4) B. R. Baker, *Cancer Chemotherapy Rept.*, **4**, 1, 1959.

(5) (a) M. P. Mertes and S. E. Saheb, *J. Heterocyclic Chem.*, **2**, 491 (1965); (b) M. P. Mertes, S. E. Saheb, and D. Miller, *ibid.*, **2**, 493 (1965); (c) T. Y. Shen, W. V. Ruyle, and R. L. Bugianesi, *ibid.*, **2**, 495 (1965); (d) M. P. Mertes, S. E. Saheb, and D. Miller, *J. Med. Chem.*, **9**, 876 (1966); (e) A. Dipple and C. Heidelberger, *ibid.*, **9**, 715 (1966).

(6) (a) R. D. Elliott, C. Temple, Jr., and J. A. Montogomery, *J. Org. Chem.*, **31**, 1890 (1966); (b) J. DeGraw, L. Goodman, B. Weinstein, and B. R. Baker, *ibid.*, **27**, 576 (1962), and references to earlier papers.

(7) (a) S. Suzuki and S. Marumo, *J. Antibiotics* (Tokyo), **A10**, 20 (1957); (b) R. J. Rousseau, L. B. Townsend, and R. K. Robins, *Biochemistry*, **5**, 756 (1966); (c) K. Tanaka, J. Sugawa, R. Nakamori, Y. Sanno, and Y. Ando, *J. Pharm. Soc. Japan*, **75**, 770 (1955); (d) J. A. Montgomery and K. Hewson, *J. Org. Chem.*, **30**, 1528 (1965); *J. Med. Chem.*, **8**, 708 (1965); (e) C. A. Salemink and G. M. Van der Want, *Rec. Trav. Chim.*, **68**, 1013 (1949); (f) R. K. Robins, J. K. Horner, C. V. Greco, C. W. Noell, and C. G. Beames, Jr., *J. Org. Chem.*, **28**, 3041 (1963).

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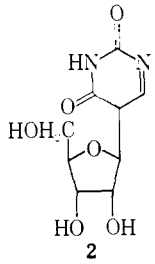
There have been no reports of synthetic deaza analogs of nucleosides where the nitrogen bonded to the sugar has been replaced by carbon. Although several C-glycosyl compounds have been found in plants,[9] the sole compounds reported in bacteria and mammals are pseudouridine (**2**) and the formycin antibiotics.[10]



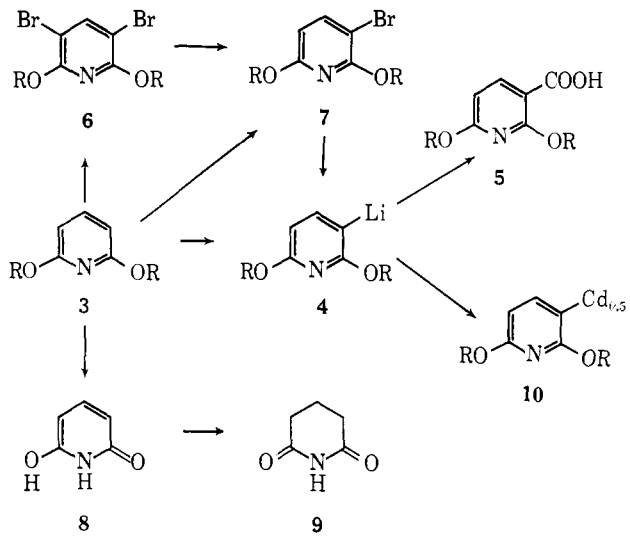
2

The synthesis of 1-deazauridine 5′-monophosphate (**1a**) and 2′-deoxy-1-deazauridine 5′-monophosphate (**1b**) requires the formation of a C–C glycosyl linkage between the pyridine portion and C_1 of the respective sugar. Appropriate protection of the oxygen functions of the sugar and the pyridine would permit the use of a route involving alkylation of the protected halosugar by the pyridine derivative. Successful application of this approach has been reported using organometallic (lithium) derivatives of pyrimidines[11] and triazines.[12] Thus, initial attempts in the synthesis of the title compounds centered on the procedure used in the synthesis of pseudouridine.[11c]

Metallations with *n*-butyllithium involving the replacement of a hydrogen atom of an aromatic system adjacent to a hetero atom are common.[13] Model reactions (Chart I) in the pyridine series showed that 2,6-dimethoxypyridine (**3a**) was converted to the 3-lithium derivative (**4a**) using *n*-butyllithium. The position and extent of metallation was established by treatment of **4a** with solid CO_2 to give a 25% yield of 2,6-dimethoxynicotinic acid (**5a**). Alternatively, **4a** was prepared *via* the halopyridine derivative. Dibromination of **3a** according to the method of den Hertog, *et al.*,[14] gave 3,5-dibromo-2,6-dimethoxypyridine (**6a**). Monodebromination of **6a** by treatment with 1 equiv of *n*-butyllithium followed by hydrolysis gave 3-bromo-2,6-dimethoxypyridine (**7a**). The latter also was obtained by low-temperature bromination of **3a**. Using the monobromo compound **7a** for metallation substantially improved the yield of **4a** since treatment with CO_2 gave 55% carboxylation (**5a**).

The relative ease of hydrogenolysis of a benzyl ether under mild conditions prompted the use of the benzyl protective group in the pyridine derivative. 2,6-Dibenzyloxypyridine (**3b**) was prepared by treatment of

CHART I



a, R = CH_3; b, R = $CH_2C_6H_5$

2,6-dichloropyridine with sodium benzyloxide. Studies on the ease of hydrogenolysis revealed that controlled reduction to 2,6-dihydroxypyridine (**8**) (2 moles of H_2) or glutarimide (**9**) (3 moles of H_2) was feasible. Following the sequence in Chart I, 2,6-dibenzyloxypyridine (**3b**) was treated with bromine and a base to give 3-bromo-2,6-dibenzyloxypyridine (**7b**) which was converted to the lithium compound (**4b**). The position of metallation was shown by carboxylation to **5b**.

Hurd and Miles[15] demonstrated in the reaction of an organolithium compound with halosugars that the reactivity of the lithium compound prohibited selective reaction at the halogen-bearing carbon. Poor yields in the pseudouridine synthesis[11c,d] also were attributed to this factor. Thus it was not unexpected that treatment of 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride[16] (**11**) with **4b** gave a multitude of products as shown by thin layer chromatography.

Shapiro and Chambers[11c] noted the desirability of using the less reactive cadmium reagent for the synthesis of pseudouridine. Coupled with the report by Hurd and Holysz[17] on the selectivity of the reaction of diphenylcadmium with halosugars, attempts were made to employ the pyridylcadmium compound. Rather than utilize the conventional method of conversion of the Grignard reagent to the cadmium compound, di-2,6-dibenzyloxypyridyl-3-cadmium (**10b**) was obtained directly by refluxing the lithium compound **4b** with cadmium chloride.

Addition of the halosugar **11b** to the cadmium compound **10b** (Chart II) followed by chromatography on silica gel and alumina gave a material that analyzed correctly (CHN) for **12**. Subsequent esterolysis of this material revealed the presence of two isomers (**12** and **13**) in the original mixture. The desired product, 3-(D-ribofuranosyl)-2,6-dibenzyloxypyridine (**14**) was obtained in 10% yield while 1,2-O-[3-(2,6-dibenzyloxypyridyl)]benzylidene-α-D-ribofuranose (**15**) was formed in 25% yield. Assignment of structure **15** was based on the elemental analysis, the nmr spectrum, and acid

(9) L. J. Haynes, *Advan. Carbohydrate Chem.*, **18**, 227 (1963).

(10) (a) F. F. Davis and F. W. Allen, *J. Biol. Chem.*, **227**, 907 (1957); (b) C. Yu and F. W. Allen, *Biochim. Biophys. Acta*, **32**, 393 (1959); (c) W. E. Cohn, *J. Biol. Chem.*, **235**, 1488 (1960); (d) R. K. Robins, L. B. Townsend, F. Cassidy, J. F. Gerster, A. F. Lewis, and R. L. Miller, *J. Heterocyclic Chem.*, **3**, 110 (1966).

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(13) (a) H. Gilman, *Org. Reactions*, **8**, 258 (1954); (b) S. V. Sunthankar and H. Gilman, *J. Org. Chem.*, **16**, 8 (1951).

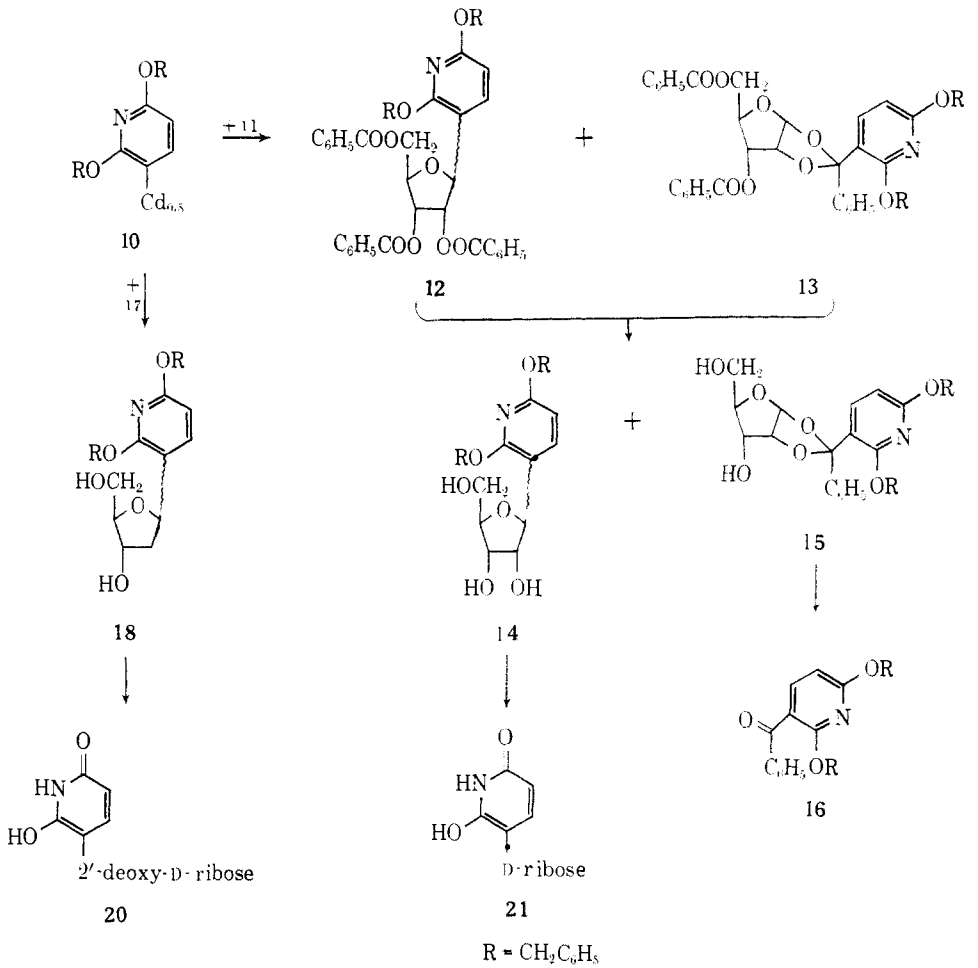
(14) H. J. den Hertog, J. P. Wibaut, F. R. Schepman, and A. van der Wal, *Rec. Trav. Chim.*, **69**, 700 (1950).

(15) C. D. Hurd and H. T. Miles, *J. Org. Chem.*, **29**, 2976 (1964).

(16) H. M. Kissman, C. Pidacks, and B. R. Baker, *J. Am. Chem. Soc.*, **77**, 18 (1955).

(17) C. D. Hurd and R. P. Holysz, *ibid.*, **72**, 2005 (1950).

CHART II



hydrolysis of the ketal **15** yielding 3-benzoyl-2,6-dibenzyloxypyridine (**16**) and a water-soluble component presumed to be ribose (positive periodate and Fehlings test).

Hurd and Holysz[17] have reported the formation of an alkylidene derivative analogous to **13** upon treatment of tetraacetyl-α-D-glucopyranosyl bromide with dibutylcadmium and dibenzylcadmium to give reaction at the C_2 acetoxy group. Coupled with the participation of the C_2 ester in controlling the anomeric configuration in the formation of purine and pyrimidine ribonucleosides,[18] the preponderence of reaction of the cadmium compound **10b** at the C_2 carbonyl in **11** to give the benzylidene **13** was not unexpected.

After treatment of the cadmium reagent **10b** with 3,5-di-O-*p*-toluyl-2-deoxy-D-ribofuranosyl chloride[19] (**17**), the resulting mixture was transesterified using sodium methoxide in methanol. Chromatography on silica gel yielded a small quantity of 6-benzyloxy-2-pyridione and the desired 3-(2-deoxy-D-ribofuranosyl)-2,6-dibenzyloxypyridine (**18**) in yields ranging up to 65%.

Cohn[10c] reported that equilibration of pseudouridine with acid gave four isomers, A_s, A_f, B, and C. Chambers and co-workers[20a] noted the presence of small quantities of the A_f isomer in addition to C, B, and A_s on base equilibration. Employing periodate oxidation the C and B isomers were shown to consist of the β- and α-ribofuranosyl structures[10c,21] and the A isomers, the anomers in the ribopyranosyl series.[22]

Similar isomerization could not be excluded in this series for the ribose (**14**) and the 2′-deoxyribose (**18**) compounds. Using 3-(2-deoxyribofuranosyl)-2,6-dibenzyloxypyridine (**18**) the furanosyl nature of the ring was established by lack of periodate consumption at pH 7 (Table I). At a pH of 1.5 slow consumption of an average 0.95 mole of periodate/mole of **18** was observed, indicating equilibration to the ribopyranose structure (**19**). Additional support for the six-membered ring structure (**19**) was derived from prior equilibration in 2 *M* HCl. After neutralization to pH 7 an average of 1.1 moles of periodate was consumed. Prior equilibration with base followed by neutralization and oxidation at pH 7 consumed 0.4 mole equiv of periodate. Thus, base equilibration results in an approximate 60:40 ratio with the ribofuranose structure (**18**) predominating.

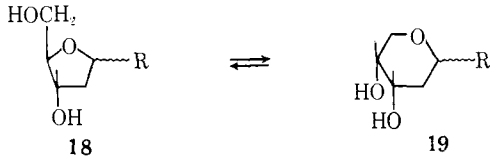
The nmr spectrum of 3-(2-deoxy-D-ribofuranosyl)-2,6-dibenzyloxypyridine (**18**) supports the assigned structure. No attempt has been made to assign the configuration at the anomeric position due to the complex pattern observed which suggests a mixture of the α and β anomers.

Hydrogenolysis of the dibenzyloxy compounds **14** and **18** was hampered by instability of the products and

(18) B. R. Baker, J. P. Joseph, R. E. Schaub, and J. H. Williams, *J. Org. Chem.*, **19**, 1786 (1954).
(19) M. Hoffer, *Ber.*, **93**, 2777 (1960).
(20) R. W. Chambers, V. Kurkov, and R. Shapiro, *Biochemistry*, **2**, 1192 (1963).
(21) A. M. Michelson and W. E. Cohn, *ibid.*, **1**, 490 (1962).
(22) R. W. Chambers and V. Kurkov, *ibid.*, **3**, 326 (1964).

Table I

Periodate Consumption by 3-(2′-Deoxyribofuranosyl)-2,6-dibenzyloxypyridine (18)



18 ⇌ 19

	Prior equilibration			
	None	None	Acid	Base
pH of IO_4^- soln	7	1.5	7	7
$[IO_4^-]/[18]$ av	0.01	0.95	1.1	0.38

the attendant problems in purification. Catalytic reduction of **14** or **18** in ethanol–hydrochloric acid followed by lyophilization, extraction, and precipitation from alcohol–ether mixtures gave the respective products 3-D-ribosyl-2,6-dihydroxypyridine (**21**) and 3-(2-deoxy-D-ribosyl)-2,6-dihydroxypyridine (**20**). Attempts to employ chromatography or crystallization for purification resulted in rapid decomposition to insoluble red materials. The instability of **20** and **21** prohibited studies to determine the cyclic structure of the sugar and the anomeric configuration at $C_{1'}$.

Several authors[23] have noted the ease of chemical oxidation of 2,6-, 2,3-, and 3,6-dihydroxypyridines to the corresponding azaquinones. Although Ames and coworkers[23b] were able to isolate 4-methyl- and 4,5-dimethyl-2,3,6-trihydroxypyridine the unsubstituted 2,3,-6-triol has not been reported. Reductive acetylation (zinc–acetic anhydride) of 2,3,6-hydroxyazaquinone is reported to yield 2,3,6-triacetoxypyridine.[23a] Solutions of 2,6-dihydroxypyridine at pH values varying from 2 to 12.5 were found to air oxidize at varying rates with alkaline and neutral oxidations proceeding in several hours. The ultraviolet absorption pattern observed for the oxidation products was dependent on the pH of the oxidation media. Ensign and Rittenberg[24] also studied this oxidation and reported that 2,6-dihydroxypyridine undergoes spontaneous oxidation to give a pigment that has the characteristics of azaquinones which arise from the chemical oxidation of trihydroxypyridines. Thus, the instability of the reduction products **20** and **21** is attributed to spontaneous air oxidation to the corresponding 2,5,6-trihydroxy compounds followed by rapid oxidation in neutral or basic media to *o*- or *p*-azaquinones.[25]

Biological Studies.—The *in vitro* studies were carried out on purified preparations of thymidylate synthetase and dihydrofolate reductase. Details of the assay method in this laboratory have been described.[26] Thymidylate synthetase was isolated according to the method of Wahba and Friedkin[27] from *E. coli* B. Dihydrofolate reductase was purified from chicken liver according to the procedure of Mathews and Huennekens.[28] Difficulties were encountered in the assay of the hydroxypyridines **8**, **20**, and **21** due to instability in the assay media and the attendant changes in absorption at 340 mμ, the wavelength used in the spectrophotometric assay for both enzymes. Thus in contrast to preliminary reports,[29] none of the compounds displayed significant inhibition. The results are summarized in Table II. Absorption of light at 340 mμ by some of the compounds prohibited studies at higher concentrations by this assay procedure. The inactivity of **20** and **21** against thymidylate synthetase was not unexpected since the 5′-phosphate is an essential feature of inhibitors of this enzyme. In addition, the anomeric configuration of **20** and **21** could not be determined because of the instability of these compounds.

Table II

Results of Enzyme Inhibition Studies

	Thymidylate synthetase[a]		Dihydrofolate reductase[b]	
Compd	Max concn tested, *M*	Inhib, %	Max concn tested, *M*	Inhib, %
8	4.2×10^{-5}	<10	1.3×10^{-3}	10
3a[c]	8.3×10^{-4}	<10		
7a[c]	4.1×10^{-4}	10		
2,3,6-Hydroxyazaquinone[d]	6.4×10^{-4}	<10		
14[e]	8.3×10^{-5}	0	6.7×10^{-5}	0
15[e]	1.7×10^{-5}	10	6.7×10^{-6}	28
21	1.5×10^{-5}	10		
18[e]	8.3×10^{-5}	0	1.3×10^{-4}	<10
20	8.3×10^{-5}	<10		

[a] 2′-Deoxyuridine 5′-monophosphate, 4.2×10^{-5} *M*; tetrahydrofolic acid, 1.8×10^{-4} *M*. [b] Reduced nicotinamide–adenine dinucleotide phosphate (NADPH), 7×10^{-5} *M*; dihydrofolic acid, 3×10^{-5} *M*. [c] Dimethyl sulfoxide was used as the solvent in both control (uninhibited) and inhibited assays. [d] Synthesized according to ref 23a. [e] Ethanol was used as the solvent in both control (uninhibited) and inhibited assays.

Inhibition of *E. coli* B/r by compounds **8**, **20**, and **21** was examined by the turbidimetric assay (550 mμ). Growth in minimal broth (synthetic) with 0.2% glucose at pH 7 was not inhibited at the following maximum concentrations: **8**, 1×10^{-5} *M*; **20**, 8×10^{-5} *M*; and **21**, 9×10^{-5} *M*. At the pH of the media **8**, **20**, and **21** would exist almost entirely as the monoanion; assays at the pH required for appreciable concentrations of the neutral species were not investigated. Instability of **8** and **21** was noted by formation of a blue pigment in the assays with an ultraviolet absorption pattern corresponding to 2,3,6-hydroxyazaquinone.[23]

Compound **15** was inactive against leukemia L1210 and nontoxic at dosage levels of 400 mg/kg.

Experimental Section

Melting points were obtained on a Thomas–Hoover Unimelt and are corrected. Ultraviolet spectra were determined on Bausch and Lomb Spectronic 505, Beckman DB, and Cary 14 spectrophotometers in ethanol unless otherwise indicated. Infrared spectra were recorded on Beckman IR5, IR8, and IR10 spectrophotometers. Nmr spectra were recorded on a Varian Associates Model A-60 spectrophotometer using Me_4Si as the internal standard. Microanalyses were performed by Drs. G. Weiler and F. B. Strauss, Oxford, England, Huffman Microanalytical Laboratories, Wheatridge, Colo., and also using an F and M Model 185 CHN analyzer.

2,6-Dimethoxynicotinic Acid (5a).—A solution of 5 g (0.036

(23) (a) J. H. Boyer and S. Kruger, *J. Am. Chem. Soc.*, **79**, 3552 (1957); (b) D. E. Annes, R. E. Bowman, and T. F. Grey, *J. Chem. Soc.*, 3008 (1953); (c) J. A. Moore and F. J. Marascia, *J. Am. Chem. Soc.*, **81**, 6049 (1959).

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(25) Professor James McChesney and Professor Ralph Adams, University of Kansas, are acknowledged for useful discussions on this point.

(26) M. P. Mertes and N. R. Patel, *J. Med. Chem.*, **9**, 868 (1966).

(27) A. J. Wahba and M. Friedkin, *J. Biol. Chem.*, **237**, 3794 (1962).

(28) C. K. Mathews and F. M. Huennekens, *ibid.*, **238**, 3436 (1963).

(29) J. Zielinski and M. P. Mertes, Abstracts, 152nd National Meeting of the American Chemical Society, New York, N. Y., Sept 1966, Abstract P22

mole) of 2,6-dimethoxypyridine (**3a**) in 100 ml of dry ether was added dropwise (15 min) to a stirred solution of 5.1 g (0.080 mole, 34 ml of a 15% solution in hexane)[36] of *n*-butyllithium in 150 ml of dry ether, under an N_2 atmosphere. The resulting amber solution was refluxed for 2 hr and at the end of this time was deep red in color. This solution was added, with stirring, to a suspension of excess solid CO_2 in dry ether. The resulting suspension was allowed to warm to room temperature and extracted with three 50-ml portions of water. The aqueous layer was acidified with 5% HCl and allowed to stand for 24 hr. At the end of this time a yellow solid had formed which, after filtration and crystallization from ethanol, yielded 1.94 g (26%) of **5a**: mp 136–137°; λ_{max} 243 mμ (ε 10,800), 288 mμ (ε 13,100).

Anal. Calcd for $C_8H_9NO_4$: C, 52.45; H, 4.95; N, 7.64. Found: C, 52.64; H, 4.82; N, 7.52.

3,5-Dibromo-2,6-dimethoxypyridine (6a).—A solution of 57 g (0.36 mole) of bromine in 75 ml of glacial acetic acid was added dropwise (1 hr) to a stirred solution of 25 g (0.18 mole) of 2,6-dimethoxypyridine (**3a**) in 75 ml of glacial acetic acid. The yellowish orange suspension was stirred for 3 hr and poured into 1 l. of water. After filtration, the resulting white solid (45 g, 85%) was dried. Crystallization from ethanol–water yielded white needles: mp 89.5–90.5°; λ_{max} 236 mμ (ε 10,700), 303 mμ (ε 9160).

Anal. Calcd for $C_7H_7Br_2NO_2$: C, 28.30; H, 2.37; N, 4.71; Br, 53.82. Found: C, 28.20; H, 2.59; N, 4.57; Br, 54.10.

3-Bromo-2,6-dimethoxypyridine (7a). A. Debromination of 3,5-Dibromo-2,6-dimethoxypyridine (6a).—A solution of 20 g (0.13 mole) of **6a** in 300 ml of dry ether was added dropwise over 2 hr to a stirred solution of 4.28 g (0.067 mole, 53 ml of a 8.0% solution in hexane) of *n*-butyllithium in 250 ml of dry ether under an N_2 atmosphere. With cooling, 125 ml of 10% HCl was added slowly (*Caution: spattering*) to the resulting solution. The layers were separated and the aqueous layer was extracted with two 50-ml portions of ether. The ether layers were combined, dried ($MgSO_4$), filtered, and evaporated *in vacuo* to yield 16 g of a deep red oil. Distillation yielded 9.8 g (69%) of **7a**: bp 115–118° (29 mm); $n^{30}D$ 1.5540; λ_{max} 229 mμ (ε 10,600), 290 mμ (ε 8300).

Anal. Calcd for $C_7H_8BrNO_2$: C, 38.55; H, 3.69; N, 6.42; Br, 36.64. Found: C, 38.55; H, 3.85; N, 6.36; Br, 36.80.

B. Bromination of 2,6-Dimethoxypyridine (3a).—A solution of 17.3 g (0.11 mole) of bromine in 300 ml of CCl_4 was added dropwise (2 hr) to a rapidly stirred suspension of 20 g (0.11 mole) of **3a** in 200 ml of CCl_4 cooled to −20 to −25° by a Dry Ice–acetone bath. After the bromine addition was complete the bath was removed and the yellow reaction mixture was allowed to warm to room temperature. After washing with 300 ml of water, the aqueous layer was extracted with two 50-ml portions of CCl_4. The organic layers were combined, dried ($MgSO_4$), filtered, and evaporated *in vacuo* to yield 21.4 g of a cloudy yellow oil which on distillation yielded 17 g (72%) of **7a**, bp 115–119° (29 mm). This compound showed infrared and ultraviolet absorption identical with that of the compound prepared by debromination of **6a**.

2,6-Dibenzyloxypyridine (3b).—With stirring, 5.2 g (0.22 g-atom) of sodium was added as small pieces to 60 ml of benzyl alcohol. After the initial exothermic reaction had subsided, the mixture was heated to 110° to facilitate the sodium consumption. When the reaction was complete 15 g (0.10 mole) of 2,6-dichloropyridine was added slowly (10 min), and the mixture was heated at 175° for 4 hr. After cooling, the resulting pale yellow mass was shaken vigorously with 500 ml of water to yield a tan crystalline solid which was collected by filtration and recrystallized from ethanol–water to yield 26.8 g (90%) of **3b** as white plates, mp 74–75°, λ_{max} 281 mμ (ε 10,200).

Anal. Calcd for $C_{19}H_{17}NO_2$: C, 78.32; H, 5.77; N, 4.80. Found: C, 77.87; H, 5.88; N, 4.71.

Reduction of 2,6-Dibenzyloxypyridine.—One gram (3.5 mmoles) of **3b** in ethanol was reduced at 3 atm with 75 mg of 5% Pd–C as catalyst. After 7 mmoles of H_2 had been consumed, the catalyst was removed by filtration and the resultant blue filtrate was evaporated *in vacuo* to yield 2,6-dihydroxypyridine (**8**) as a reddish yellow solid. The solid was washed with 20 ml of ether yielding 110 mg (28%): mp 187–189° (lit.[34] 195 and 184–185°); $\lambda_{max}^{H_2O}$ 234, 320 mμ (lit.[34] $\lambda_{max}^{H_2O}$ 234, 320 mμ).

Repeating the above procedure but using 540 mg of 5% Pd–C at 2 atm of hydrogen and continuing until H_2 uptake ceased yielded 330 mg of glutarimide (**9**) (83%).

3-Bromo-2,6-dibenzyloxypyridine (7b).—A solution of 13.7 g (0.086 mole) of bromine in 200 ml of CCl_4 was added dropwise (2 hr) to a rapidly stirred suspension of 40 g of finely ground anhydrous K_2CO_3 and 25 g (0.086 mole) of 2,6-dibenzyloxypyridine (**3b**) in 500 ml of CCl_4 maintained at room temperature by occasional cooling. After the bromine addition was complete, the reaction mixture was washed several times with water. The water layers were combined and extracted with 100 ml of CCl_4. The organic layers were combined, dried ($MgSO_4$), filtered, and evaporated *in vacuo* to give a pale yellow oil which crystallized from absolute ethanol to yield 29 g (90%) of **7b** as small white needles: mp 57–59°; λ_{max} 233 mμ (ε 91000), 294 mμ (ε 10,100).

Anal. Calcd for $C_{19}H_{16}BrNO_2$: C, 61.63; H, 4.35; N, 3.78; Br, 21.58. Found: C, 61.79; H, 4.21; N, 3.89; Br, 22.00.

2,6-Dibenzyloxynicotinic Acid (5b).—A solution of 0.5 g (1.35 mmoles) of 3-bromo-2,6-dibenzyloxypyridine (**7b**) in 25 ml of absolute ether was added dropwise to a stirred solution of 0.22 g (5.2 mmoles; 22 ml of a 15% solution in hexane) of *n*-butyllithium in 25 ml of absolute ether under an N_2 atmosphere. After stirring at room temperature for 1 min, excess solid CO_2 was added to the bright red solution. The resulting cloudy solution was stirred until it warmed to room temperature and treated with 50 ml of water. The layers were separated and the aqueous layer was washed with 10 ml of ether. The combined water layers were acidified with 10% HCl. A solid precipitated and was collected (400 mg, 92%): crystallization from methanol yielded **5b** as small white needles: mp 131.5–132.5°; λ_{max} 246 mμ (ε 3800), 290 mμ (ε 4900).

Anal. Calcd for $C_{20}H_{17}NO_4$: C, 71.63; H, 5.10; N, 4.17. Found: C, 72.15; H, 5.24; N, 4.29.

3-(D-Ribofuranosyl)-2,6-dibenzyloxypyridine (14) and 1,2-O-[3-(2,6-Dibenzyloxy)pyridyl]benzylidene-α-D-ribofuranose (15).—A solution of 3.55 g (9.5 mmoles) of 3-bromo-2,6-dibenzyloxypyridine (**7b**) in 25 ml of absolute ether was added in one portion to a rapidly stirred solution of 0.70 g of *n*-butyllithium (11 mmoles, 7.8 ml of a 9.0% solution in hexane) in 25 ml of absolute ether in an N_2 atmosphere. After 30 min of stirring at room temperature, 1.10 g (6 mmoles) of anhydrous $CdCl_2$, dried *in vacuo* for 4 hr at 110°, was added in one portion to the deep red solution of the lithium salts. The resulting suspension was refluxed gently for 2.5 hr, 35 ml of ether was distilled from the mixture, and one-half of a solution of 3.0 g (6.3 mmoles) of 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride[18] (**11**) in 50 ml of anhydrous toluene was added in one portion. The remaining ether was distilled and the other 25 ml of the chlorosugar solution was added. The resulting green mixture was refluxed for 11 hr.

After cooling, the mixture was hydrolyzed with ice water and acidified with a minimal amount of acetic acid (20 ml), enough for complete solution of the solid. The layers were separated and the aqueous layer was extracted with toluene. The combined organic layers were washed twice with a saturated $NaHCO_3$ solution and finally with water. The toluene solution was dried ($MgSO_4$), filtered, and evaporated *in vacuo* to yield 6.0 g of a deep red syrup.

Thin layer chromatographic examination of the mixture on silica gel revealed four major spots. A portion of the reaction mixture (4 g) was chromatographed on a column of 200 g of silica gel (0.05–0.20 mm) using acetone–cyclohexane (1:9 and 1:4) for elution collecting 100-ml fractions. Fractions 12–14 contained 942 mg of what appeared to be the expected nucleoside (35% yield). Fraction 12 was further purified for elemental analysis by chromatography on 30 g of Woelm alumina (activity 1) using 5% ethyl acetate in benzene for elution, taking 10-ml fractions. The third 10-ml fraction contained 121 mg of a clear gum which was further chromatographed on 50 g of silica gel using 15% acetone in cyclohexane for elution again taking 10-ml fractions. After a forerun of 135 ml was collected, the fifth fraction was evaporated and dried *in vacuo* at 45° for 1 hr to yield 34 mg of a clear gum: λ_{max} 231 mμ (ε 37,300), 281 mμ (ε 5200). This material was a mixture of the two empirical isomers **12** and **13**.

Anal. Calcd for $C_{26}H_{27}NO_6$: C, 73.45; H, 5.07; N, 1.90. Found: C, 73.20; H, 5.27; N, 1.72.

A portion of the crude reaction mixture containing **12** and **13** (not chromatographed) (26.8 g) in 200 ml of anhydrous benzene–methanol (1:1) was treated with 10 ml of sodium methoxide in

[36] Foote Mineral Co., New Johnsonville, Tenn.

methanol, prepared by the addition of two small chips of Na. The red solution was allowed to stand at room temperature overnight. The pH of the solution was adjusted from 11 to 6.8 with Dowex 50W-X4 (50–100 mesh H^+ form) cation-exchange resin. The mixture was filtered free of the resin and evaporated *in vacuo* to yield 30 g of a deep red oil.

After noting four major spots on tlc, the reaction mixture (30 g) was chromatographed on 400 g of silica gel (0.05–0.20 mm) using $CHCl_3$ and methanol in $CHCl_3$ (1% and 3%) for elution taking 500-ml fractions. Fractions 6–13 were evaporated *in vacuo* to yield 3.1 g (25%) of **15** as an orange solid. This material was recrystallized from ethanol to yield white fluffy needles: mp 107–109°; λ_{max} 235 mμ (ε 9200), 284 mμ (ε 7800).

Anal. Calcd for $C_{31}H_{29}NO_5$: C, 70.57; H, 5.54; N, 2.65. Found: C, 70.71; H, 5.69; N, 2.83.

Fractions 21–24 were evaporated *in vacuo* and recrystallized from benzene to yield 900 mg (10%) of an orange amorphous solid. An analytical sample was prepared by recrystallization from benzene to yield **14** as small white needles: mp 184–185°; λ_{max} 231 mμ (ε 15,300), 284 mμ (ε 10,200); $[\alpha]^{25}D$ −10° (*c* 2.38, EtOH).

Anal. Calcd for $C_{24}H_{25}NO_6$: C, 68.07; H, 5.95; N, 3.30. Found: C, 67.87; H, 5.97; N, 3.47.

Fractions 25–27 were combined and evaporated *in vacuo* to yield 200 mg of a deep red gum which defied crystallization. This material was not characterized further.

3-Benzoyl-2,6-dibenzyloxypyridine (16) *via* Acid Hydrolysis of 1,2-O-[3-(2,6-Dibenzyloxy)pyridyl]benzylidene-α-D-ribofuranose (15).—A solution of 0.1 g (0.19 mmole) of **15** in 100 ml of ethanol–water (1:1) was treated with 1 ml of HCl. The solution was refluxed for 1.5 hr. Upon cooling to room temperature 0.067 g (90%) of 3-benzoyl-2,6-dibenzyloxypyridine (**16**) crystallized as white fluffy needles. Recrystallization from ethanol–water gave a melting point of 117–117.5°; λ_{max} 255 mμ (ε 10,300), 309 mμ (ε 10,000); ir, 6.05 μ (conjugated C=O); nmr, four benzylic protons at 5.25 and 5.40 ppm, two pyridyl proton doublets centered at 6.5 and 8.9 ppm, 15 aromatic protons in the 7–7.5 ppm region.

Anal. Calcd for $C_{26}H_{21}NO_3$: C, 78.96; H, 5.35; N, 3.54. Found: C, 78.85; H, 5.45; N, 3.53.

After separation of the ketone **16** the filtrate gave a positive periodate test and a positive Fehlings test indicating ribose as the other product of the reaction.

3-D-Ribosyl-2,6-dihydroxypyridine (21).—An ethanol solution of 119 mg of **14** (0.28 mmole) and approximately 50 mg of 5% Pd–C was reduced at atmospheric pressure until 0.56 mmole of H_2 was absorbed. Lyophilization, after filtration, gave a dark residue which was dissolved in ethanol and precipitated with ether twice to give 15 mg (20%) of **21** as a tan powder: mp 190°, darkens 170°; $\lambda_{max}^{0.1\ M\ HCl}$ 320 mμ (ε 4600); 0.1 *M* NaOH, 235 mμ (ε 7500), 335 mμ (ε 6800).

Anal. Calcd for $C_{10}H_{13}NO_6$: C, 49.38; H, 5.39; N, 5.77. Found: C, 49.18; H, 4.92; N, 5.41.

3-(2-Deoxy-D-ribofuranosyl)-2,6-dibenzyloxypyridine (18).—A solution of 22.2 g (0.06 mole) of 3-bromo-2,6-dibenzyloxypyridine (**7b**) in 100 ml of absolute ether was added in one portion to a rapidly stirred solution of 37.5 ml of a 1.6 *M* solution of *n*-butyllithium in hexane (0.06 mole) in 100 ml of absolute ether, under an N_2 atmosphere. After 30 min of stirring at room temperature 5.50 g of anhydrous $CdCl_2$ (0.03 mole) was added in one portion to the orange solution of lithium salts. The resulting mixture was refluxed gently for 1.5 hr. After most of the ether was distilled, a solution of 10.45 g (0.027 mole) of 3,5-di-*p*-toluyl-2-deoxy-D-ribofuranosyl chloride[19] (**17**) in 100 ml of anhydrous toluene was added in one portion to the Cd salts. The remaining ether was distilled and the resulting mixture was refluxed for 4.5 hr and finally stirred at room temperature overnight.

The mixture was hydrolyzed with 125 ml of ice–water and acidified with acetic acid. The layers were separated and the aqueous layer was extracted with $CHCl_3$. The toluene and $CHCl_3$ extracts were each washed separately with saturated $NaHCO_3$ solution and with water. The organic layers were dried ($MgSO_4$), filtered, and evaporated *in vacuo* to yield a red oil. Without further purification this oil was dissolved in 300 ml of anhydrous benzene–methanol (1:1), treated with a few chips of Na, and allowed to stand overnight at room temperature. The pH of the resulting solution was adjusted from 11 to 6.8 with Dowex 50W-X4 (50–100 mesh, H^+ form) cation-exchange resin. The mixture was filtered free of the resin and evaporated *in vacuo* to yield an amber oil. The oil, subjected to tlc examination, showed five major spots.

The oil was dissolved in a minimum amount of $CHCl_3$ and chromatographed on 400 g of silica gel (0.05–0.20 mm) using $CHCl_3$ and methanol in $CHCl_3$ (1%) for elution. After elution of the first fraction (pyridines and methyl benzoate) a small quantity of 6-benzyloxy-2-pyridone was isolated, mp 128–129°; this was characterized by ir (6.05 μ, C=O) and nmr (a 5.85-ppm doublet for the 3 proton, a 6.3-ppm doublet for the 5 proton, J = 9 cps, a benzylic methylene singlet at 5.12 ppm, and the remainder of the aromatic protons in the 7.2–7.5-ppm region.

Anal. Calcd for $C_{12}H_{11}NO_2$: C, 71.62; H, 5.51; N, 6.96. Found: C, 71.78; H, 5.75; N, 6.96.

The third band of material collected consisted of 7.08 g of **18** (65%) as a pale amber glass that solidified on exposure to air. Recrystallization from benzene–Skelly B gave **18** as white needles: mp 89–91°; λ_{max} 230 mμ (ε 15,500), 285 mμ (ε 9700); $[\alpha]^{25}D$ −25° (*c* 3.5, EtOH).

Anal. Calcd for $C_{24}H_{25}NO_5$: C, 70.74; H, 6.18; N, 3.44. Found: C, 70.52; H, 6.22; N, 3.30.

The pyridyl protons appear as doublets at 7.7 (H_4) and 6.4 (H_3) ppm; the benzylic methylenes are two singlets at 5.35 ppm while the sugar proton assignments compare favorably with reported deoxynucleosides. The anomeric proton (C_1'-H) in **18** was observed as a multiplet at about 5.2 ppm overlapping the benzylic methylene signal. Further evidence for this assignment was observed in the analogous dimethoxy compound where the anomeric proton signal as a multiplet is confirmed at 5.2 ppm.

3-(2-Deoxy-D-ribosyl)-2,6-dihydroxypyridine (20).—The dibenzyloxy compound **18** (322 mg, 0.8 mmole) was dissolved in 15 ml of ethanol containing 2 ml of HCl. This was added to a prereduced suspension of 100 mg of 10% Pd–C in 50 ml of ethanol. After the theoretical quantity of H_2 was absorbed the suspension was filtered and the light yellow filtrate was lyophilized to give a tan solid. With continuous flushing with dry N_2 this was dissolved in methanol and precipitated by cooling and the addition of ether to give a light yellow powder. Repeated lyophilization, solution, and precipitation of the mother liquors gave a total of 49 mg (22%) of **20**: mp 185° dec; $\lambda_{max}^{0.1\ M\ HCl}$ 323 mμ (ε 4600); 0.1 *M* NaOH, 236 mμ (ε 7500), 334 mμ (ε 6600).

Anal. Calcd for $C_{10}H_{13}NO_5 \cdot CH_3OH$: C, 50.96; H, 6.61; N, 5.40. Found (two samples): C, 51.20, 51.05; H, 6.28, 6.12; N, 5.53, 5.28.

The presence of methanol was confirmed by the nmr spectrum of **20** in D_2O. Nmr studies of **20** in d_6-DMSO-D_2O showed one aromatic proton at 7.5 ppm corresponding to the pyridine H_4 and poorly resolved patterns for the sugar protons. The absence of the aromatic H_5 proton in the nmr spectrum of **20** was expected since 2,6-dihydroxypyridine (**8**) in d_6-DMSO having the H_3 and H_5 doublet at 6.5 ppm and the H_4 triplet centered at 8 ppm rapidly exchanges the 3 and 5 protons with D_2O and degenerates to a singlet for the 4 proton at 8.0 ppm.

Periodate Oxidation Studies on 3-(2-Deoxy-D-ribofuranosyl)-2,6-dihydroxypyridine (18).—The results of periodate oxidation of **18** are listed in Table I; standard procedures[31] were used. A stock solution of 0.086 *M* **18** in ethanol was used. Equilibration in either acid or base was followed by neutralization to pH 7, evaporation, and extraction with ethanol to 0.086 *M* concentration. Aliquots of the stock solution were oxidized with sodium metaperiodate. Oxidations were followed by titration with standardized arsenite and iodine solutions and corrected for consumption of periodate by the blank.

Acknowledgments.—The authors wish to acknowledge Mrs. William Riggs for the results of the enzyme studies and Professor Delbert Shankel and Miss Judy Kleinberg for assistance in the growth inhibition studies. Special acknowledgement is due the following undergraduate students for their assistance in preparing synthetic intermediates: Miss Ann Bowman and Messrs. Duane Miller and Larry Hare.

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