TABLE I
ULTRAVIOLET ABSORPTION

	λ, mμ (ε × 10⁻³)					
	0.1 N HCl		pH 7		0.1 N NaOH	
Compd	max	min	max	min	max	min
1a	280 (14.3)	261 (9.0)	281 (13.2)	261 (7.3)	286 (13.1)	253 (4.8)
	247.5 (12.6)	237.5 (11.3)	248 (7.9)	242 (7.6)		
1b	291 (12.9)	271 (8.8)	281 (15.5)	243 (6.8)	281 (15.5)	243 (6.8)
	254 (12.1)	238 (8.0)	263 sh (10.9)		263 sh (10.9)	
1c	287 (17.3)	250 (1.9)			285 (15.9)	256 (3.7)
2a	278 (12.4)	241 (2.5)	267.5 (9.2)	248 (6.9)	282 (8.1)	253 (3.7)
			232 (8.5)		230 sh (8.3)	
2b	282.5 (15.8)	243 (2.9)	271 (13.0)	247.5 (9.2)	271 (13.0)	247.5 (9.2)
			239 (9.6)	228 (9.1)	239 (9.6)	228 (9.1)

by neutralization with glacial AcOH. The precipitate was washed with H_2O and dried to give 300 mg (55%) of 1c monohydrate. An analytical sample was prepared by recrystallization from 1 *N* NaOH by acidification with AcOH; mp >300° dec. *Anal.* ($C_{10}H_{13}N_5O \cdot H_2O$) C, N; H: calcd, 6.37; found, 5.92.

2-Hydroxy-4-(3-methyl-2-butenylamino)pyrimidine (2a).—4-Methylthiouracil[16] (1.5 g, 10.5 mmoles), γ,γ-dimethylallylamine (5.0 g, 59 mmoles), and EtOH (5.0 ml) were refluxed for 2.5 hr. The precipitate which formed on cooling was washed with petroleum ether (30–60°) and recrystallized from 95% EtOH and dilute H_2SO_4 to give 1.82 g (95%) of **2a**·0.5H_2SO_4. A sample for analysis was recrystallized twice from EtOH–H_2O and dried; mp 229–230°. *Anal.* ($C_9H_{13}N_3O \cdot 0.5H_2SO_4$) C, H, N.

2-Hydroxy-4-(3-methyl-2-butenylamino)-1-β-D-ribofuranosylpyrimidine (2b).—1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-4-thiouracil[17] (2.0 g, 4.3 mmoles) was refluxed with γ,γ-dimethylallylamine (10 g, 118 mmoles) for 90 min. The excess amine was removed under vacuum to give a brown oil. The oil was dissolved in 10 ml of EtOH and 300 ml of Et_2O was added. The pale tan oil which separated was shown by thin layer chromatography (silica gel; GF_{254}, $CHCl_3$–MeOH 3:1) to be essentially free of N-(3-methyl-2-butenyl)benzamide. Final separation was carried out on a silica gel column (2.5 × 18 cm, Fisher silica gel 100–200 mesh, grade 923) by elution with $CHCl_3$–MeOH 3:1. The solvent was removed under vacuum to give an amorphous and somewhat unstable product. *Anal.* ($C_{34}H_{31}N_3O_4$) H, N; C: calcd, 54.01; found, 53.02.

A tetrabenzoyl derivative of **2b** was prepared by heating 100 mg of **2b** with an excess of benzoyl chloride in pyridine at 60° until tlc (silica gel GF_{254}, C_6H_6–Et_2O 2:1) showed a single spot at R_f ~0.50. The product was poured over ice and gave a light tan oil. The oil was triturated with H_2O and allowed to stand for a few days. The needles which formed were recrystallized twice from EtOH; mp 193–194°. *Anal.* ($C_{32}H_{37}N_3O_5$) C, H, N.

(16) Prepared according to the method of T. Ueda and J. J. Fox, *J. Med. Chem.*, **6**, 697 (1963).

(17) J. J. Fox, D. Van Praag, I. Wempen, I. L. Doerr, L. Cheong, J. E. Knoll, M. L. Eidinoff, A. Bendich, and G. B. Brown, *J. Am. Chem. Soc.*, **81**, 178 (1959).

Nucleosides. XLVII. Syntheses of Some N⁴-Substituted Derivatives of 1-β-D-Arabinofuranosylcytosine and -5-fluorocytosine[1]

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The clinical usefulness of 1-β-D-arabinofuranosylcytosine (**4**) in the treatment of acute leukemias and lymphomas has been demonstrated,[2a] but various pharmacological studies[2b,c] have shown that this drug is rapidly deaminated to the inactive nucleoside, 1-β-D-arabinofuranosyluracil (**1**). It has been shown previously that alkylation of the exocyclic amino function of cytosine nucleosides, such as 2′-deoxy-5-fluorocytidine produced a marked decrease of the susceptibility of such compounds to deamination by bacterial deaminases.[3] It was further shown[4] that **4** also serves as a substrate for bacterial deaminases. The 5-fluoro analog (**4b**, R″ = H)[5] was also effective against several mouse leukemias, but this analog, too, is degraded by human liver or mouse kidney deoxycytidine deaminases to **1b**, R = H.[6] These results[6,7] suggested the synthesis of N⁴-substituted derivatives of **4a** and **4b** as potential chemotherapeutic agents and/or as deoxycytidine deaminase inhibitors. The present report describes the synthesis of several of these N⁴-substituted derivatives selected for biological evaluation.

The syntheses of N⁴-substituted arabinosylcytosine nucleosides (**4**) were accomplished by the thiation process[5,8] which involved thiation of a suitably protected nucleoside (**1**) with phosphorus pentasulfide in pyridine to give the 4-thiones (**2**) followed by alkylation to the 4-methylthio analogs (**3**). Treatment of **3** with various nucleophiles (*e.g.*, NH_2OH, NH_2NH_2, CH_3NH_2) gave the desired products (**4**) (Scheme I).

Though the synthesis of **2b** (R = $COCH_3$) had been reported,[5] its preparation by thiation of **1b** was rather difficult and several retreatments with P_2S_5 were necessary to drive the reaction to completion. Moreover, during the extended thiation-reaction time, some of the acetyl-protecting groups were apparently lost and **2b** could not be obtained in pure form. Although crude **2b**, thus obtained, was satisfactory for subsequent conversions, it was hoped that benzoyl blocking groups

(1) This investigation was supported in part by funds from the National Cancer Institute (Grant CA 08748).

(2) (a) R. Talley and V. K. Viackevicius, *Blood*, **21**, 352 (1963); E. S. Henderson and P. J. Burke, *Proc. Am. Assoc. Cancer Res.*, **6**, 26 (1965); R. W. Carey and R. R. Ellison, *Clin. Res.*, **13**, 337 (1965); K. P. Yu, J. P. Howard, and B. D. Clarkson, *Proc. Am. Assoc. Cancer Res.*, **7**, 78 (1966); R. R. Ellison, J. F. Holland, T. Silver, J. Bernard, and M. Boiron, *Proc. 9th Intern. Cancer Congr., Tokyo, 1967*, in press; (b) R. Papac, W. A. Creasey, P. Calabresi, and A. D. Welch, *Proc. Am. Assoc. Cancer Res.*, **6**, 50 (1965); (c) G. W. Camiener and C. G. Smith, *Biochem. Pharmacol.*, **14**, 1405 (1965).

(3) I. Wempen, R. Duschinsky, L. Kaplan, and J. J. Fox, *J. Am. Chem. Soc.*, **83**, 4755 (1961).

(4) L. I. Pizer and S. S. Cohen, *J. Biol. Chem.*, **235**, 2387 (1960).

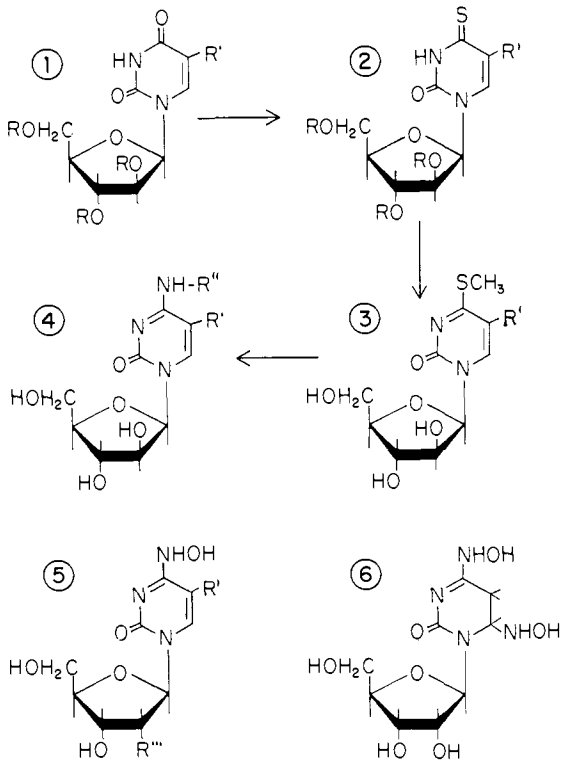
(5) J. J. Fox, N. Miller, and I. Wempen, *J. Med. Chem.*, **9**, 101 (1966).

(6) M. R. Dollinger, J. H. Burchenal, W. Krise, and J. J. Fox, *Biochem. Pharmacol.*, **16**, 689 (1967).

(7) G. W. Camiener, *ibid.*, in press.

(8) J. J. Fox, D. Van Praag, I. Wempen, I. L. Doerr, L. Cheong, J. E. Knoll, M. L. Eidinoff, A. Bendich, and G. B. Brown, *J. Am. Chem. Soc.*, **81**, 178 (1959).

SCHEME I



a series, R'=H R=acetyl, benzoyl, or H
b series, R'=F R''=OH, NH_2, CH_3 or $COCH_3$
R'''=H or OH

(known to be more stable under thiating conditions) would give higher yields of pure **2b** (R = COPh). The available 1-(2,3,5-tri-*O*-benzoyl-β-D-arabinosyl)-5-fluorouracil[9] (**1**) was employed. Better yields of **2b** (R = COPh) were obtained in the thiation step, although in this case also, more than one P_2S_5 treatment was required.[10] Compound **2b** (R = COPh) was deesterified in alkali and S-alkylated to **3b**. This methylation step (**2b** → **3b**) was used because, as reported previously,[5] the methylthio group is more easily replaced by nucleophiles than a 4-thione group. Thus, milder conditions may be employed for the conversion of **3b** → **4b** without concomitant replacement[3] of the 5-fluorine atom. Treatment of **3b** with hydroxylamine in ethanol or with anhydrous hydrazine (99%) at room temperature yielded **4b** (R'' = OH or NH_2, respectively) in good yield. The methylamino analog **4b** (R'' = CH_3) was obtained by treatment of **3b** with liquid methylamine in a sealed bomb at room temperature.

Similarly, the N^4-hydroxy, -amino, and -methyl derivatives of 1-β-D-arabinofuranosylcytosine (**4a**, R'' = OH, NH_2, and CH_3, respectively) were synthesized from **1a** (R = $COCH_3$) by a series of reactions essentially similar to those described in the conversion of **1b** → **4b**. The N^4-acetyl derivative of **4a** was prepared by a procedure analogous to that used for selective N^4-acylation of cytidine.[11]

For comparative studies with deoxycytidine deaminase,[6] several N^4-hydroxycytosine nucleosides were prepared in which the sugar moiety was D-ribose or 2-deoxy-D-erythropentose ("2-deoxy-D-ribose") instead of D-arabinose. The compounds selected for these studies were the N^4-hydroxy derivatives (**5**) of cytidine, 2'-deoxycytidine, 2'-deoxy-5-fluorocytidine, and 2'-deoxy-5-methylcytidine.

N^4-Hydroxycytidine and 2'-deoxy-N^4-hydroxy-5-methylcytidine had been synthesized previously[8] by hydroxylamination of 4-thiouridine and 4-thiothymidine. The reported procedure[8] was modified by the use of milder conditions and the yields have been improved. In the case of 2'-deoxy-5-fluoro-N^4-hydroxycytidine (**5b**), it was advantageous to S-methylate 4-thio-5-fluorouridine[3] with diazomethane (quantitative yield) and to treat the resulting 4-methylthio derivative with hydroxylamine.

During the treatment of 4-thiouridine (4.5 mmoles) with a tenfold excess of anhydrous hydroxylamine in 40 ml of methanol containing 1 ml of water, a "bishydroxylamine" derivative formed as indicated by the disappearance of uv absorption at 328 mμ and the concomitant rise of a strong peak at 222 mμ. The white solid obtained from this reaction gave elemental analyses consistent with **6** obtained by Brown and Schell[12] from cytidine. Acid treatment of **6** gave the known N^4-hydroxycytidine (**5a**, R''' = OH). The formation of a bishydroxylamino derivative (**6**) in the reaction of hydroxylamine with a 4-thio precursor has not been noted previously.[13]

For practical considerations, more facile preparations of N^4-hydroxycytidine were made when considerably higher dilutions (4 mmoles of the thione/250 ml of methanol) were employed and hydroxylamine was regenerated *in situ* from the hydrochloride salt. Under these conditions, the "bis" intermediate (**6**) was not isolated. That **6** was an intermediate in those high-dilution reactions was shown by spectral monitoring of the reaction mixture (appearance of a strong maximum at 222 mμ which is converted rapidly in acid to the N^4-hydroxycytidine spectrum with a maximum at 280 mμ).

When a dilute solution of 4-thiothymidine[8] was treated with hydroxylamine in anhydrous methanol, a high yield of **5** (R' = CH_3, R''' = H) was obtained.

(9) The authors are indebted to Dr. A. Nussbaum of Hoffmann-La Roche, Inc., Nutley, N. J., for a generous gift of this compound.

(10) Apparently the difficulty in thiating the 4-oxo group of **1b** (R = $COCH_3$ or COPh) is encountered only with the 5-fluoro-*arabino* nucleosides. *ribo* analogs of **1** thiate normally. This unexpected characteristic of **1b** is not clearly understood and is worthy of investigation.

(11) K. A. Watanabe and J. J. Fox, *Angew. Chem. Intern. Ed. Engl.*, **5**, 579 (1966).

(12) D. M. Brown and P. Schell, *J. Chem. Soc.*, 208 (1965).

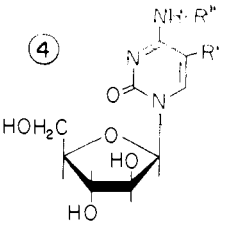
(13) These data do not prove that the conversion of 4-thiouridine to N^4-hydroxycytidine proceeded *exclusively via* intermediate **6**. Kinetic experiments by Lawley[14] showed that a significant portion of the reaction of cytosine and 2'-deoxycytidine with NH_2OH under aqueous conditions proceeds by direct displacement of the exocyclic amino group by NH_2OH. This latter mechanism had been postulated by Janion and Shugar[15] for the reaction of aqueous NH_2OH at pH 6.5 with 5- and 6-alkylated cytosines and with certain 4-alkoxy-2-pyrimidinones. The conversion of the S-methylated arabino nucleosides (**3**) to their N^4-hydroxylamino analogs (**4**, R'' = OH) reported in the present paper (*vide supra*) were performed at 70–80° in anhydrous EtOH, conditions which differ from those of the other investigators.[12,14,15] It is known that the 4-methylmercapto groups of 2-pyrimidinones and their nucleosides are easily displaced by nucleophiles,[16] which suggests that the conversion of **3** to **4** (R'' = OH) probably proceeded *via* direct displacement of the 4 substituent by NH_2OH.

(14) P. D. Lawley, *J. Mol. Biol.*, **24**, 75 (1967).

(15) C. Janion and D. Shugar, *Acta Biochim. Polon.*, **12**, 337 (1965).

(16) Y. Mizuno, M. Ikehara, and K. A. Watanabe, *Chem. Pharm. Bull.* (Tokyo), **10**, 653 (1962).

TABLE I
SPECTROPHOTOMETRIC AND pK_a[a] DATA OF 1-β-D-ARABINOFURANOSYLCYTOSINES



R′	R″	pH	λ_{max}, mμ	ϵ_{max}	λ_{min}, mμ	ϵ_{min}	Cationic pK_a[c]
H[b]	H	1 *N* HCl	213, 281	9,750, 13,200	241	1770	4.2
		7.7	sh 230, 270	8,690, 9,490	249	6320	
H	OH	1 *N* HCl	221, 282	8,430, 14,570	244	3100	2.4
		4.97	237, 270	13,420, 6,870	262	6610	
H	NH_2	1 *N* HCl	215, 281	8,440, 13,150	242	2620	4.4
		7.70	sh 230–235, 273	7,300, 10,330	247.5	6790	
H	CH_3	1.0	218, 282	8,280, 13,510	242	2190	3.9
		7.70	236, 271	8,470, 10,915	248	7300	
H	$COCH_3$	1 *N* HCl	213, sh 230, 312	11, 100, 8,900, 17,650	261	1470	~1.4
		6.17	214, 247, 298	16,360, 14,550, 8,810	227, 270	6000, 3520	
F	H	1 *N* HCl	221, 290.5	10,300, 11,900	246	1160	2.3
		5–7	235, 280	7,860, 8,240	225, 257.5	7550, 5240	
F	OH	3 *N* HCl	224, 290	9,090, 13,530	250	3580	0.7
		7.70	236, 272	11,280, 8,980	257	8070	
F	NH_2	1 *N* HCl	214, 288	9,060, 11,124	252	2900	2.7
		4.97	242, 281	7,640, 10,670	255	7190	
F	CH_3	1 *N* HCl	218, 290	9,860, 13,000	248	1970	2.0
		4.97	240, 278	8,730, 10,290	257	7630	

[a] Only the basic dissociations were determined. [b] E. R. Walwick, W. K. Roberts, and C. A. Dekker, *Proc. Chem. Soc.*, 84 (1959), report the following: at pH 2, maxima at 212, 279 (ε 9800 and 13,400), minimum at 240 (ε 1200), pK_a = 4.1. [c] Data taken from ref 5.

Spectrophotometric monitoring of the reaction mixture did not reveal the presence of any any "bishydroxylamino" intermediate nor could such an intermediate be isolated from the reaction mixture.

The results with 4-thiouridine and 4-thiothymidine described above point to the marked effect which 5-alkylation exerts on the course taken for the displacement of the 4-thione by hydroxylamine in these nucleosides. These effects are probably akin to observations[12,14,15] made for the reaction of cytosines and 5-methylcytosines with hydroxylamine.

The ultraviolet absorption data for the cationic and neutral species[17] of the N^4-substituted derivatives **4a** and **4b**, are shown in Table I, together with the corresponding data for the unsubstituted analogs. The fluorinated compounds (R′ = F) exhibit a bathochromic shift of *ca.* 8 mμ in the spectral pattern relative to their 5-unsubstituted analogs (R′ = H). A similar shift has been reported previously[3] for 1-β-D-ribofuranosyl-5-fluorocytosine, 2′-deoxy-5-fluorocytidine, and some of their derivatives compared with cytidine, 2′-deoxycytidine, and the corresponding derivatives.

In addition, the presence of a fluorine atom in the 5 position of these 1-β-D-arabinofuranosylcytosine nucleosides has a marked base-weakening effect (*ca.* 1.8 units) on the cationic pK_a values of the corresponding 5-unsubstituted analogs (see the last column of the table). The degree to which the pK_a values are decreased, again, is in agreement with that found in a comparison with the D-ribosyl- and 2′-deoxy-D-erythropentosyl analogs noted above (*ca.* 1.8 units).[3] The marked decrease in the basicity of **4b** (R″ = H) (pK_a = 2.3) *vs.* that of cytidine (pK_a = 4.1) is reflected in the failure of attempts[18] to N^4-acetylate the former compound by the procedure of Watanabe and Fox.[11] It is also apparent from an examination of the cationic pK_a values within each series (R′ = H or F) that, in accord with previous observations,[8,15] 4-hydroxylamino nucleosides are weaker bases than their corresponding cytosine analogs.

Screening Data.[19]—In preliminary experiments, N^4-acetyl-1-β-D-arabinofuranosylcytosine administered intraperitoneally at a dose of 200 mg/kg daily for ten doses caused a 50% increase in life span (ILS) in mice with leukemia L1210 without any evidence of toxicity. In the same experiment, **4a** (R″ = H), administered at 10 mg/kg daily for ten doses produced an ILS of 105%. A detailed study of the biological and biochemical effects produced by the other nucleoside analogs described in this paper has been reported recently.[5]

Experimental Section[20]

1-(2,3,5-Tri-*O*-benzoyl-β-D-arabinosyl)-5-fluoro-4-thiouracil (2b, R = COPh).—Compound **1b** (R = COPh,[9] 20 g, 0.035 mole), in 800 ml of pyridine was treated with 15.5 g (0.07 mole) of P_2S_5, and the well-stirred suspension was heated to reflux. As solution occurred, 0.9 ml of H_2O was added *cautiously* until a semiturbid, oily suspension was obtained. The reaction mixture

(17) Data for the anionic species was not determined since, with the exception of N^4-methyl derivatives, these N^4-substituted cytosine nucleosides exhibit alkali instability to a certain degree.

(18) Unpublished experiments by Dr. R. J. Cushley of this laboratory.

(19) The authors are indebted to Dr. J. H. Burchenal and his associates of this institute for these data.

(20) All melting points were taken on a Thomas–Hoover capillary melting point apparatus and are corrected. Microanalyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich., and by Dr. F. Scheidel of Hoffmann-La Roche Co., Nutley, N. J. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

was refluxed for 4 hr. Progress of the thiation was monitored by examination of small aliquots. Repeated evaporation of these samples with 50% EtOH removed all the pyridine. The residual syrups were dissolved in EtOH and the spectrophotometric ratio 270/333 mμ was obtained. This ratio should be 0.45–0.55 for completely thiated product. A higher ratio indicates incomplete thiation and the necessity for the addition of a second charge of P_2S_5 (*ca.* 10 g). The reaction mixture was cooled somewhat, and the addition was made cautiously to prevent foaming. The thiation was usually completed in *ca.* 10 hr and with the addition of at least one extra portion of thiating agent. The black reaction mixture was cooled, and the pyridine was decanted from a solid which was washed well with CH_2Cl_2 and discarded. The combined solvents were evaporated *in vacuo* to a thin syrup which was poured into a well-stirred H_2O–ice slurry. Stirring was continued for 0.5 hr until the precipitate had become completely granular. The somewhat colloidal suspension was filtered, and the collected precipitate was washed throroughly with H_2O. The precipitate was then dissolved in CH_2Cl_2, and the solution was dried (Na_2SO_4). The solvent was removed *in vacuo*, and the residual, thin syrup was poured with stirring into 600 ml of hot EtOH. The solution was allowed to cool slowly and finally refrigerated overnight. The bright yellow precipitate was filtered, washed with cold EtOH, and finally with Et_2O. The yield of pure product was 17 g (83%), mp 187–189°. Evaporation of the mother liquor yielded a further crop which was recrystallized from EtOH, 1.9 g, mp 182–186°. *Anal.* Calcd for $C_{30}H_{23}FN_2O_8S$: N, 4.74; S, 5.43. Found: N, 5.05; S, 5.23.

1-β-D-Arabinofuranosyl-5-fluoro-4-thiouracil (2b, R = H).[21]—Compound **2b** (R = COPh, 30 g, 0.05 mole), 400 ml of MeOH, 200 ml of H_2O, and 200 ml of 1 *N* NaOH were stirred at room temperature for 3 hr. The reaction mixture was treated with portions of Dowex 50 (H^+) resin until a neutral pH was attained. The resin was filtered and washed with two 100-ml portions of MeOH. The combined filtrates were evaporated *in vacuo* to a thick syrup which was dissolved in hot H_2O and cooled slowly. The granular precipitate was filtered, triturated thoroughly with CH_2Cl_2, and air-dried; 13 g (93%), mp 172–175°. A portion, recrystallized from hot EtOH, melted at 177–179°. *Anal.* ($C_9H_{11}N_2FO_5S$) C, H, N, F, S.

1-β-D-Arabinofuranosyl-5-fluoro-4-hydroxylamino-2(1H)-pyrimidinone (4b, R″ = OH).—A solution of NH_2OH[22] (0.05 mole) in 100 ml of absolute EtOH was added to a stirred solution of **3b** (1.5 g, 0.005 mole)[5] in 20 ml of EtOH. The clear solution was stirred and heated at 70–75° for 2 hr. The solvent was evaporated *in vacuo*. Hot absolute EtOH was added to the residue, some insolubles were filtered, and the filtrate was reduced to *ca.* 20 ml and cooled. The crystalline precipitate (1.3 g, mp 153°, effervescence) was recrystallized from a minimum of EtOH, and the pure product (0.9 g, mp 157–158°, effervescence) was obtained as a hemialcoholate, $[\alpha]^{23}D$ +101° (*c* 0.4, H_2O). *Anal.* ($C_9H_{12}FN_3O_6 \cdot 0.5C_2H_5OH$) C, H, N.

1-β-D-Arabinofuranosyl-5-fluoro-4-hydrazino-2(1H)-pyrimidinone (4b, R″ = NH_2).—A stirred solution of **3b** (274 mg, 0.001 mole) in 5 ml of EtOH was treated with 1 ml of EtOH containing 0.05 g of anhydrous NH_2NH_2 (99.9%), and the reaction mixture was left at room temperature for 20 hr. The solvents were removed *in vacuo* (bath ~25°). The residual orange syrup was dissolved in 5 ml of MeOH and treated with 10 ml of Et_2O previously saturated at 0° with HCl. The HCl salt precipitated as yellow needles, 350 mg, dec pt 178°. The product was recrystallized by solution in MeOH and addition of Et_2O to incipient turbidity. An analytical sample of the product had mp 178–179° (effervescence), $[\alpha]^{23}D$ +140° (*c* 0.3, H_2O). *Anal.* ($C_9H_{13}FN_4O_5 \cdot HCl$) C, H, N.

1-β-D-Arabinofuranosyl-5-fluoro-4-methylamino-2(1H)-pyrimidinone (4b, R″ = CH_3).—Compound **3b** (500 mg) was placed in a glass liner of a bomb tube and chilled in a Dry Ice bath. $MeNH_2$ gas was introduced into the chilled liner until *ca.* 15 ml of the liquid amine had condensed. The bomb was sealed, left at room temperature for 20 hr, cooled, vented, and opened, and the excess $MeNH_2$ was evaporated under a stream of air. The residue was dissolved in hot EtOH, and the solvent was evaporated *in vacuo*. This process was repeated several times to remove any unreacted $MeNH_2$. During this repetitive evaporation, some crystallization occurred. The small amount of granular precipitate was filtered and washed with EtOH; mp 218–219°. The filtrate was evaporated to a syrup which resisted further crystallization. Therefore, the solid and syrup were dissolved in MeOH and treated with EtOH–HCl. The precipitate was crystallized from MeOH–Et_2O. The yield of the hydrochloride was 0.7 g, mp 180–181° (effervescent), $[\alpha]^{23}D$ +151° (*c* 0.5, H_2O). *Anal.* ($C_{10}H_{14}FN_3O_5 \cdot HCl$) H, F, N; C: calcd, 38.53; found, 39.09.

1-β-D-Arabinofuranosyl-4-methylthio-2(1H)-pyrimidinone (3a).—Compound **1a**,[23] (R = $COCH_3$, 12.5 g, 0.0034 mole) was dissolved in 200 ml of reagent grade pyridine and the efficiently stirred solution was treated with 14.3 g (0.064 mole) of P_2S_5. The temperature of the reaction mixture was raised until reflux began. Water (5 drops) was added *cautiously*, and the amber reaction mixture was stirred and refluxed for 3 hr. The cooled reaction mixture was filtered, and the precipitate was washed with a little pyridine and discarded. The filtrate was evaporated *in vacuo* to a thick syrup which was treated with 50% EtOH and the solvent was evaporated. This addition and reevaporation process was repeated several times, thereby removing most of the pyridine. The granular, amber residue was extracted with $CHCl_3$, and the extract was filtered to remove insolubles. The $CHCl_3$ filtrate was evaporated to a syrup which was dissolved in 50% EtOH and filtered and the filtrate was evaporated. The residue was crystallized from hot 75% EtOH. The product was obtained in two crops, 12.3 g, mp 110–115°. Recrystallization from hot C_6H_6–EtOH gave platelets, mp 113–117° (sintered at *ca.* 100°). This product, without further purification, was used directly for the methylation reaction.

Compound **2a** (R = $COCH_3$, 12.3 g, 0.032 mole) was stirred and heated with 14.2 g (0.1 mole) of MeI in a mixture of 100 ml of MeOH and 50 ml of H_2O. NaOH (1 *N*, 32 ml) was added dropwise. After the addition was complete, the solution was stirred for 1 hr and then neutralized to pH 5 with dilute AcOH. The MeOH was evaporated *in vacuo*, and the aqueous solution was chilled. A precipitate of long needles, 7.7 g (84% based on **1a**), was obtained. A portion, recrystallized from H_2O, gave pure **3a**, mp 123–124°. *Anal.* ($C_{10}H_{14}N_2O_5S$) N, S.

1-β-D-Arabinofuranosyl-4-hydroxylamino-2(1H)-pyrimidinone (4a, R″ = OH).—A stirred solution of **3a** (2 g, 0.0073 mole) in 50 ml of EtOH was treated with 100 ml of EtOH containing 0.05 mole of NH_2OH.[22] The mixture was stirred overnight at room temperature. A second 100-ml portion of EtOH containing 0.05 mole of NH_2OH was added, and the reaction mixture was refluxed for 3 hr. The solvent was removed *in vacuo*, and the residue was dissolved in a little H_2O and applied to a column of Dowex 50 (H^+), previously washed free of uv-absorbing material. The column was washed with H_2O until all starting material was removed. The column was eluted with 1 *N* NH_4OH, and the product-containing fractions were combined and evaporated *in vacuo* to dryness. The residue was crystallized from hot EtOH; yield 0.8 g, mp 130–131° (effervescent). One recrystallization from EtOH afforded an analytical sample of the hemialcoholate, mp 131–132° (effervescence), $[\alpha]^{23}D$ +101° (*c* 0.3, H_2O). *Anal.* ($C_9H_{13}N_3O_6 \cdot 0.5C_2H_5OH$) C, H, N.

1-β-D-Arabinofuranosyl-4-hydrazino-2(1H)-pyrimidinone (4a, R″ = NH_2).—To a solution of **3a** (1.32 g, 0.0048 mole) in 30 ml of EtOH was added slowly 0.35 ml of $NH_2NH_2 \cdot H_2O$. The reaction mixture was refluxed for 3 hr and cooled. Colorless needles, precipitated; 1.15 g, mp 219–220°. Recrystallization from 80% EtOH yielded a pure sample, mp 219–220°, $[\alpha]^{23}D$ +158° (*c* 0.4, H_2O). *Anal.* ($C_9H_{14}N_4O_5$) C, H, N.

1-β-D-Arabinofuranosyl-4-methylamino-2(1H)-pyrimidinone (4a, R″ = CH_3).—Compound **3a** (1.0 g, 0.0036 mole) was placed in a well-cooled, glass-lined bomb and *ca.* 10 ml of liquid $MeNH_2$ was added. The bomb was sealed and left at room temperature for 20 hr. The excess $MeNH_2$ was evaporated in a stream of air. Upon addition of EtOH to the residue, crystallization occurred. The crude product, 1.1 g, mp 258–259° (effervescence), was recrystallized from 60% EtOH; colorless needles, 0.9 g, mp 264–

(21) This compound was isolated as a syrup in ref 5.

(22) The NH_2OH was prepared *in situ* as follows. The correct molar amount of $NH_2OH \cdot HCl$ was dissolved in a minimum of hot MeOH and allowed to cool to room temperature. One drop of phenolphthalein was added to the solution which was stirred and treated with a freshly prepared solution of NaOMe in a minimum of MeOH until a faint pink color persisted. (Over-neutralization must be avoided since the 4-hydroxylamino nucleosides are rather unstable to alkali.) The precipitated NaCl was removed, and the free NH_2OH solution was used immediately.

(23) D. M. Brown, A. Todd, and S. Varadarajan, *J. Chem. Soc.*, 2388 (1956).

265° dec,[24] $[\alpha]^{23}D$ +150° (c 0.5, H_2O). *Anal.* ($C_{10}H_{15}N_3O_5$) C, H, N.

4-Acetylamino-1-β-D-arabinofuranosyl-2(1H)-pyrimidinone (4a, R = $COCH_3$).[25]—The HCl salt of 1-β-D-arabinofuranosylcytosine (**4a**, R'' = H, 1.1 g) was dissolved in H_2O and added to a Dowex 1 (OH^-) column. After elution with ~2.5 l. of H_2O, the eluate was concentrated to dryness *in vacuo*. The fine crystalline, free nucleoside (880 mg) was dissolved in 100 ml of MeOH and refluxed with 0.9 ml of Ac_2O for 5 hr. Additional 0.9-ml portions of Ac_2O were added during the reflux period at hourly intervals. The reaction mixture was separated from a small amount of insoluble material, and the filtrate was concentrated *in vacuo* to 10 ml and treated with Et_2O. A crystalline product was obtained; 0.95 g (92%), mp ~170°. Recrystallization from hot EtOH gave pure product, mp 194–195°. Uv-absorption properties (see Table I) were generally similar to those for N^4-acetylcytidine.[11] *Anal.* ($C_{11}H_{15}N_3O_6$) C, H, N.

5,6-Dihydro-4,6-dihydroxylamino-1-β-D-ribofuranosyl-2(1H)-pyrimidinone (6).—To a solution of 1.18 g (0.0045 mole) of 4-thiouridine[8,26] in 40 ml of MeOH containing 1 ml of H_2O was added 1.5 g (0.045 mole) of anhydrous NH_2OH.[27] The mixture was left for 1 hr at room temperature. An immediate evolution of H_2S occurred. After 1 hr, the uv absorption maximum of the thione peak at 328 mμ had disappeared with a corresponding rise of a new maximum at 226 mμ. The reaction mixture was evaporated *in vacuo* to a thin syrup which was treated with a large volume of Et_2O. A flocculent precipitate thus obtained was decanted through a filter and washed with Et_2O. The solid, crystallized from hot EtOH, afforded 110 mg, mp 170–171°.[28] This compound had a single, selective, uv, absorption maximum at 223 mμ. On treatment with 1 *N* HCl, the short-wavelength maximum rapidly disappeared with the concurrent appearance of a new maximum at 280 mμ. This behavior is essentially identical with that of the "bis" hydroxylamino compound reported previously.[12] *Anal.* ($C_9H_{16}N_4O_7$) C, H; N: calcd, 19.17; found, 18.73.

After removal of the "bis" compound, the mother liquor was treated with HCl and, after subsequent neutralization, afforded a product which resembled in all respects the known N^4-hydroxycytidine.[8]

4-Hydroxylamino-1-β-D-ribofuranosyl-2(1H)-pyrimidinone (5a, R', R''' = OH).—To 1.06 g (0.004 mole) of 4-thiouridine[8,26] in 50 ml of MeOH was added NH_2OH[22] (0.04 mole) in MeOH (200 ml). The mixture was held at room temperature for 80 min after which time the uv absorption at 328 mμ had completely disappeared, and a new absorption maximum at 272 mμ had appeared. The MeOH was removed *in vacuo* and EtOH was added and removed *in vacuo* three times. The residue was taken up in 75 ml of cold EtOH, the insoluble material was removed, and the filtrate was taken to dryness *in vacuo*. HCl (36 ml, 1 *N*) was added, and the mixture was heated on a steam bath for 6 min.[29] The acid was removed *in vacuo*, and the residue was taken to dryness *in vacuo* six times, three times with 25 ml of C_6H_6, and three times with 25-ml portions of EtOH. The resulting residue was dissolved in 50 ml of cold EtOH, and the product was precipitated by the addition of 200 ml of Et_2O. The solution (in EtOH) and reprecipitation were repeated to obtain a pale, straw-colored powder, 661 mg (55%), mp 182–183° dec, with darkening at 181°. The HCl salt thus obtained has spectral data which are essentially identical with those previously reported.[12]

1-(2-Deoxy-β-D-ribofuranosyl)-5-fluoro-4-methylthio-2(1H)-pyrimidinone.[30]—To a solution of 1.6 g (0.006 mole) of 2'-deoxy-5-fluoro-4-thiouridine[3] in MeOH was added excess CH_2N_2 in Et_2O. The reaction mixture was allowed to stand 1 hr and taken to dryness *in vacuo*. The crystalline product was recrystallized from MeOH and afforded an essentially quantitative yield of pale yellow needles, mp 141.5–143°, shown to be a single component by the (*n*-BuOH–H_2O, 86:14). *Anal.* ($C_9H_{13}FN_2O_4S$) C, H, N.

1-(2-Deoxy-β-D-ribofuranosyl)-5-fluoro-4-hydroxylamino-2(1H)-pyrimidinone (5, R' = F; R'' = OH; R''' = H).—A solution of 3.6 g (0.013 mole) of the S-methylated precursor in 200 ml of MeOH was treated with MeOH–NH_2OH[22] (0.13 mole), and the mixture was left at room temperature for 18 hr. The course of reaction was monitored by the disappearance of the uv absorption at 315 mμ. The solution was taken to dryness *in vacuo*. EtOH was added to the residue in three consecutive 50-ml portions, evaporating the solution to dryness each time. The residue was taken up in cold EtOH (100 ml), and the insolubles were removed by filtration. The filtrate was concentrated to *ca.* 10 ml and EtOAc was added followed by petroleum ether. The amorphous, white precipitate which resulted was purified by repeating the solution and reprecipitation step. Attempts to obtain a crystalline compound were unsuccessful. The product (1.2 g) was hygroscopic and had no definite melting point. Uv-absorption properties of the compound are in 6 *N* HCl, maxima at 219 and 290 mμ (ε 7660, 10,590), minimum at 248 mμ (ε 2040); at pH 7, maxima at 234 and 267 mμ (ε 9780, 7740), minimum at 255 mμ (ε 6920). *Anal.* ($C_9H_{12}FN_3O_5 \cdot 0.5H_2O$) C, H, N.

1-(2-Deoxy-β-D-ribofuranosyl-4-hydroxylamino-5-methyl-2(1H)-pyrimidinone (5, R' = CH_3; R'' = NHOH; R''' = H).—A solution of 3.4 g (0.013 mole) of 4-thiothymidine[8] and NH_2OH[22] (0.13 mole) in MeOH (200 ml) was heated at 38° for 5 hr. The completion of the reaction was determined by the absence of uv absorption in the 334-mμ region and cessation of H_2S evolution. The mixture was evaporated to dryness *in vacuo* and 50 ml of EtOH was added and evaporated *in vacuo*. The addition and evaporation were repeated three times. The resulting white residue was taken up in cold EtOH (100 ml), and the insoluble material was removed. To the filtrate was added 5 ml of a solution of HCl in EtOH (saturated at 0°) and 50 ml of Et_2O. Crystallization occurred slowly. The compound was recrystallized from EtOH–Et_2O. The yield was 3.4 g (88%), mp 166° dec. The uv-absorption properties are essentially identical with those previously reported for the free nucleoside.[8]

(24) A melting point of 257–260° for the crude, free base was reported by J. H. Hunter, U. S. Patent 3,116,282 (Dec 31, 1963).

(25) The synthesis of this compound was performed by Dr. Naotaka Yamaoka of these laboratories.

(26) This compound has been reported as a crystalline solid by N. K. Kochetkov, E. I. Budowsky, V. N. Shibaev, G. I. Yeliseeva, M. A. Grachev, and V. P. Denushkin, *Tetrahedron*, **19**, 1207 (1963).

(27) C. D. Hurd, *Inorg. Syn.*, **1**, 87 (1939).

(28) The melting point of the unrecrystallized compound is reported in ref 12 as 130–135°.

(29) No attempt was made to isolate the "bis" compound. However, the filtrate from the acid treatment did exhibit a nonultraviolet-absorbing component on a thin layer chromatogram (*n*-BuOH–H_2O 86:14) which was visualized by spraying with a FeCl₃ solution. The resulting pink spot has been reported as characteristic of the "bis" hydroxylamino derivatives.[12] The monohydroxylamino derivative exhibits a blue spot when treated with the same reagent.[12]

(30) This compound was previously reported[31] as a crude intermediate which was not characterized.

(31) T. Ueda and J. J. Fox, *J. Med. Chem.*, **6**, 697 (1963).

Nucleosides. XLVIII. Synthesis of 1-(5-Deoxy-β-D-arabinosyl)cytosine and Related Compounds[1]

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In the course of biochemical and biological studies[2,3] on analogs of 1-β-D-arabinofuranosylcytosine (*ara*-C), it became necessary to synthesize 5'-deoxy-*ara*-C (**9**, see Scheme I) as a possible substrate and/or inhibitor of deoxycytidine deaminase present in human liver or mouse kidney homogenates. This paper describes the synthesis of 5'-deoxy-*ara*-C by two routes from 5'-deoxyuridine by use of anhydro nucleoside intermediates.

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(2) M. R. Dollinger, J. H. Burchenal, W. Kreis, and J. J. Fox, *Biochem Pharmacol.*, **16**, 689 (1967).

(3) I. Wempen, N. Miller, E. A. Falco, and J. J. Fox, *J. Med. Chem.*, in press.