Preparation of a Number of 5-Butylpyrimidine Nucleosides†

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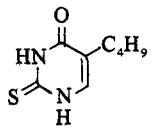
The condensation of the bis(trimethylsilyl) derivatives of 5-butyluracil and of 5-butyl-2-thiouracil with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose or with the related ribofuranosyl chloride by several methods is described. The resulting 2′,3′,5′-tri-*O*-benzoyl-5-butyluridine and 2′,3′,5′-tri-*O*-benzoyl-5-butyl-2-thiouridine can be efficiently thiated using phosphorus pentasulfide in dioxane to give the related tribenzoates of 5-butyl-4-thiouridine and 5-butyl-2,4-dithiouridine. Debenzoylation of these substances gives the 5-butyl derivatives of uridine, 2- and 4-thiouridines, and 2,4-dithiouridine. In addition, the reaction of 2′,3′,5′-tri-*O*-benzoyl-5-butyl-4-thiouridine with ammonia, methylamine, and dimethylamine gives 5-butylcytidine and its N^4-methyl and N^4-dimethyl derivatives. Similarly, amination of 2′,3′,5′-tri-*O*-benzoyl-5-butyl-2,4-dithiouridine gives 5-butyl-2-thiocytidine. Physical and spectroscopic data for all these compounds are described. The antiviral activity of the various products is also discussed.

Two recent papers have described the synthesis and antiviral activity of a series of 5-alkylpyrimidines and 5-alkylpyrimidine nucleosides.[1] The most interesting compounds were found to be 5-butyluracil (**2**) and, in particular, 5-butyluridine (**6b**), both of which showed activity against both DNA and RNA viruses. It was, in fact, suggested that 5-butyluridine showed both a greater activity and broader antiviral spectrum than did 5-fluorodeoxyuridine. This report prompted us to undertake the synthesis of a number of other 5-butylpyrimidine nucleosides in both the uridine and cytidine series, this work being summarized in the present paper.

A key intermediate for the present series of compounds is 5-butyl-2-thiouracil (**1**) which was prepared by minor modifications of the method of Burckhalter and Scarborough.[2] Hydrolysis of the thio function to give 5-butyluracil (**2**) was readily achieved according to the general procedure of Johnson and Hemingway.[3] Alternatively, treatment of **1** with phosphorus pentasulfide in dioxane converted it smoothly into 5-butyl-2,4-dithiouracil (**3**). Both with this compound and with the nucleosides to be described later, the use of dioxane rather than pyridine as the solvent for the thiation of pyrimidines as suggested by Falco, *et al.*,[4] appears to offer considerable advantage.

For the preparation of nucleosides from **1**, **2**, and **3**, these compounds were converted into their bis(trimethylsilyl) derivatives (**4a-c**) by reaction with hexamethyldisilazane in the presence of a catalytic amount of ammonium sulfate[5] and purified by distillation.



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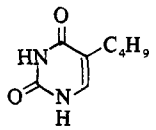

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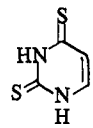

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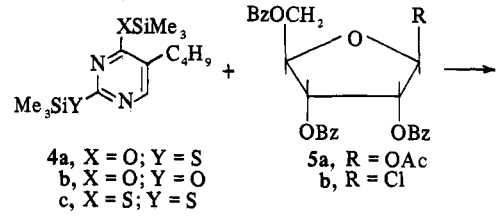
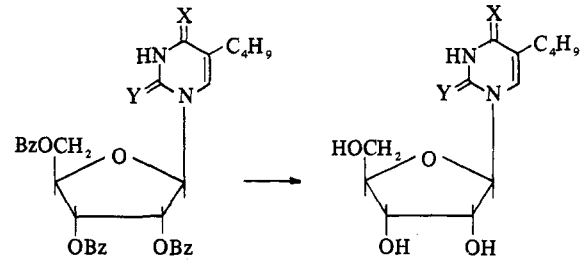
The coupling of **4b** with 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl chloride (**5b**) in the presence of mercuric chloride and mercuric oxide according to the general method of Wittenburg[6] gave crystalline 2′,3′,5′-tri-*O*-benzoyl-5-butyluridine (**6b**) in a yield of 85%. The identical material was also prepared from **5b** and bis(5-butyluracil)mercury but in this case the yield of crystalline product was only 42%. The latter method was previously used by Muraoka, *et al.*,[1a] for the preparation of **6b** in an undisclosed yield. On the other hand, we were unsuccessful in several efforts to prepare **6b** from **4b** and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**5a**) in 1,2-dichloroethane at room temperature in the presence of stannic chloride.[7] As will be seen later, this type of reaction was used with great success with closely related pyrimidine bases and the present failure is difficult to explain. In view of the highly successful preparation of **6b** by the Wittenburg method no attempts were made to use more forcing conditions.



4a, X = O; Y = S
b, X = O; Y = O
c, X = S; Y = S

5a, R = OAc
b, R = Cl



6a, X = O; Y = S
b, X = O; Y = O
c, X = S; Y = S
d, X = S; Y = O

7a, X = O; Y = S
b, X = O; Y = O
c, X = S; Y = S
d, X = S; Y = O

In contrast to the above result, the condensation of 5-butyl-*O*,*S*-bis(trimethylsilyl)-2-thiouracil (**4a**) with **5a** in 1,2-dichloroethane in the presence of roughly 0.6 equiv of stannic chloride went smoothly at room temperature and crystalline 2′,3′,5′-tri-*O*-benzoyl-5-butyl-2-thiouridine (**6a**) was isolated in 57% yield. Also, quite unlike the reactions of **4b**, we have found that condensation of **4a** with **5b** in the presence of mercuric chloride and mercuric oxide proceeds in only low yield and **6a** could be isolated by preparative tlc in only 10-15% yield. There is thus a clear preference for the Wittenburg procedure for the preparation of the 2-thionucleoside **6b** and for the procedure of Niedballa and Vorbrüggen for the preparation of **6a**.

The attempted condensation of 5-butyl-2,4-bis(trimethylsilylthio)pyrimidine (**4c**) with **5a** in the presence of stannic chloride was unsuccessful. Accordingly, the desired product (**6c**) was prepared instead by thiation of **6a** with phosphorus pentasulfide in dioxane, a reaction that gave

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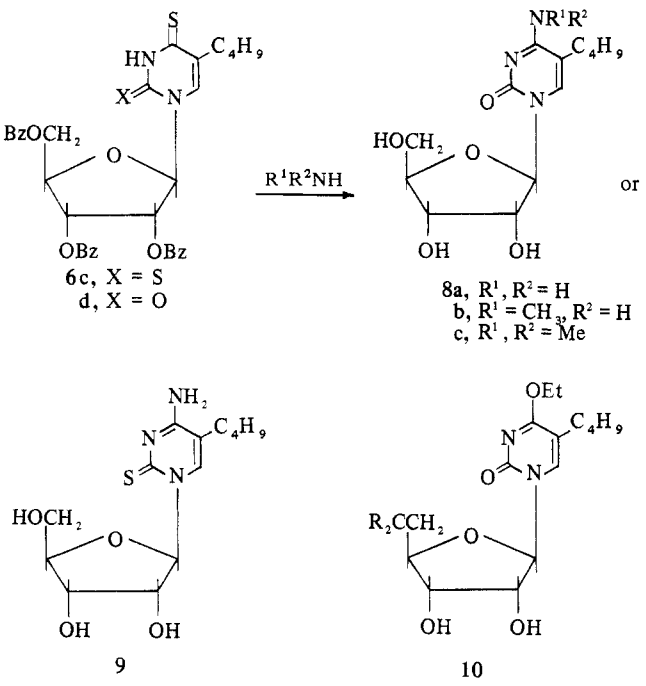
Table I. Chemical Shifts for Reported Compounds[c]

Compd	Solvent	$C_1'H$	$C_2'H$	$C_3'H$	$C_4'H$	$C_5'aH$	$C_5'bH$	C_6	Other
1	DMSO-d_6							7.30 (s)	12.1 (br s, NH)
2	DMSO-d_6							7.14 (s)	10.7 (br s, NH)
3	DMSO-d_6							7.32 (s)	12.5 (br s, NH)
6a	$CDCl_3$	7.4-7.7[a]	5.80 (dd)	5.96 (dd)	4.82 (m)	4.71 (dd)	4.97 (dd)	7.4-7.7[a]	7.4-7.7 (m, 11, Ar, $C_1'H$, C_6H), 8.0-8.3 (m, 6, Ar)
6b	$CDCl_3$	6.40 (d)	5.74 (dd)	5.91 (dd)	4.5-5 (m)	4.5-5 (m)	4.5-5 (m)	7.08 (s)	8.69 (br s, NH), 7.2-7.6 (m, 9, Ar) 7.8-8.2 (m, 6, Ar)
6c	$CDCl_3$	7.2-7.6[a]	5.73 (dd)	5.87 (dd)	4.78 (m)	4.67 (dd)	4.90 (dd)	7.2-7.6[a]	7.2-7.6 (m, 11, Ar, $C_1'H$ and C_6H) 7.9-8.2 (m, 6, Ar)
6d	$CDCl_3$	6.36 (d)	5.74 (dd)	5.91 (dd)	4.75 (m)	4.62 (dd)	4.86 (dd)	7.09 (s)	7.3-7.6 (m, 9, Ar), 7.85-8.2 (m, 6, Ar)
7a	Py-d_5	7.44 (d)	4.88 (m)	4.88 (m)	4.67 (m)	4.22 (dd)	4.39 (dd)	8.81 (s)	
7b	Py-d_5	6.77 (d)	4.87 (d)	4.87 (d)	4.60 (m)	4.12 (dd)	4.28 (dd)	8.31 (s)	
7c	Py-d_5	7.25 (d)	4.86 (m)	4.86 (m)	4.68 (m)	4.22 (dd)	4.39 (dd)	8.90 (s)	
7d	Py-d_5	6.67 (m)[b]	4.87 (m)	4.87 (m)	4.64 (m)	4.17 (dd)	4.40 (dd)	8.52 (s)	
8a	Py-d_5-D_2O	6.66 (d)	4.80 (m)	4.80 (m)	4.63 (m)	4.18 (dd)	4.34 (dd)	8.35 (s)	
8b	Py-d_5-D_2O	6.71 (d)	4.85 (m)	4.85 (m)	4.64 (m)	4.18 (dd)	4.34 (dd)	8.31 (s)	3.10 (s, 3, NMe)
8c	Py-d_5-D_2O	6.69 (d)	4.84 (m)	4.84 (m)	4.64 (m)	4.19 (dd)	4.34 (dd)	8.43 (s)	2.94 (s, 6, NMe_2)
9	Py-d_5-D_2O	7.64 (d)	4.6-5.1 (m)	4.6-5.1 (m)	4.6-5.1 (m)	4.26 (dd)	4.40 (dd)	8.94 (s)	
10	$CDCl_3$	6.58 (d)	5.71 (dd)	5.92 (dd)	4.85 (m)	4.63 (dd)	4.79 (dd)	7.2-7.6[a]	1.32 (t, 3, EtO), 4.42 (q, 2, EtO)

[a] Superimposed upon benzoyl protons and confirmed by decoupling of $C_2'H$. [b] Virtual coupling to $C_2'H$ and $C_3'H$. In DMSO-d_6 $C_1'H$ becomes a doublet, $J_{1'2'}$ = 4 Hz at 5.73 ppm. [c] In addition, each compound showed the 5-butyl group as a 3-proton triplet (CH_3) at 0.70-0.92 ppm, a 4-proton multiplet at 0.9-1.7 ppm, and a broadened triplet (pyrimidine-CH_2) at 2.0-2.9 ppm.



crystalline **6c** in 85% yield. In the same way, thiation of **6b** gave 5-butyl-2′,3′,5′-tri-*O*-benzoyl-4-thiouridine (**6d**), also in a yield of 85%.

An alternative activation of the 4 position *via* conversion to the 4-chloro derivative by reaction with thionyl chloride and dimethylformamide in chloroform failed.[8] Instead of the desired 4-chloronucleoside, the 4-ethoxy derivative (**10**) was isolated in 41% yield. The ethoxyl group presumably arose from ethanol in the chloroform in spite of the fact that this solvent had been passed through activated alumina immediately prior to use. In view of the successful thiation reactions this route was not explored further.

Removal of the benzoyl groups from the blocked 5-butylpyrimidine nucleosides **6a-6d** was readily achieved *via* brief treatment with sodium methoxide giving free 5-butyluridine (**7b**), 5-butyl-2-thiorudine (**7a**), 5-butyl-4-thiouridine (**7d**), and 5-butyl-2,4-dithiouridine (**7c**) as crystalline compounds in excellent yields.

The availability of the blocked 4-thiopyrimidine nucleosides **6c** and **6d** has also made possible the preparation of 5-butylcytidine (**8a**) and several of its analogs. Thus treatment of **6d** and of **6c** with methanolic ammonia at 100° gave 5-butylcytidine (**8a**) and 5-butyl-2-thiocytidine (**9**) which were isolated in yields of 70 and 35% by chromatography on Bio-Rad AG-1 X 2 (OH^-) resin according to Dekker.[9] During preparation of **8a** as above a second product was isolated in 10% yield and proved to be N^4-methyl-5-butylcytidine (**8b**), which was identical in every way with the compound prepared in 93% yield from **6d** and methanolic methylamine. The origin of the methyl group in this minor by-product remains obscure. In a similar way, the condensation of **6d** with methanolic dimethylamine gave 5-butyl-N^4-dimethylcytidine (**8c**) in 36% yield, together with considerable **7d**.

It is generally recognized that the condensation of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**5a**) or of the corresponding ribosyl chloride (**5b**) with trimethylsilyl pyrimidines, by the methods of Wittenburg[6] or of Niedballa and Vorbrüggen,[7] leads almost exclusively to the corresponding β-nucleosides. In the present work the products of such condensations (**6a, 6b**) were obtained in highly crystalline forms and in high yields. The nmr spectra of these compounds (see Tables I and II) or of the 4-thionucleosides (**6c** and **6d**) derived from them do not permit a direct confirmation of the presumed β configuration since the values of $J_{1',2'}$ were consistently 6 Hz. Following removal of the benzoyl groups from these substances, however, the resulting free nucleosides (**7a, c, 8a, b, c,** and **9**) all showed values of $J_{1',2'}$ of 2 Hz or less, a figure which allows, with reasonable assurance, assignment of the β configuration.‡ The value of $J_{1',2'}$ for 5-butyluridine itself is 3 Hz, a somewhat less certain figure, but the β configuration for this compound is confirmed by its ORD spectrum which showed a simple positive Cotton effect centered at 280 nm and typical of pyrimidine β-nucleosides.[11]

It may be noted from Table I that the presence of an anisotropic 2-thio function leads to strong deshielding of $C_1'H$

‡ A value of $J_{1',2'}$ of less than 3.5 Hz is generally consistent with a β configuration for ribonucleosides. A value of 1 Hz or less is, however, highly desirable for unequivocal assignments. See Tolman, *et al.*[10]

Table II. First-Order Coupling Constants in Hertz (see Table I)

Compd	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4',5'a}$	$J_{4',5'b}$	$J_{5'a,5'b}$
6a	6	6	3	3	1	11
6b	6	6	3	*a*	*a*	*a*
6c	6	6	3	3	1	11
6d	6	6	3	3.5	1	11
7a	2	*a*	*a*	1.5	2	12
7b	3	0	3	2.5	2.5	12
7c	0.5	*a*	*a*	2	2	12
7d	3[b]	*a*	*a*	2	2.5	11.5
8a	2	*a*	*a*	2	2	12
8b	2	*a*	*a*	2	2	12
8c	2	*a*	*a*	3	2	12
9	0.5	*a*	*a*	2	2	12
10	6	6	3.5	3	3	12

[a]Not resolved. [b]Spacing between the outer lines of an apparent triplet. In DMSO-d_6 $J_{1',2'}$ = 4 Hz.

in compounds **7a**, **7c**, and **9** (δ 7.25-7.64) relative to their 2-oxo counterparts (δ 6.66-6.77). Other examples of this effect have been noted.[12-14] The presence of the 2-thio function also appears to consistently lead to a downfield shift of the C_6-proton of the uracil ring. Thus, the 2-thio compounds (**7a**, **7c**, and **9**) all show C_6H as singlets at 8.81-8.94 ppm while in the 2-oxo nucleosides (**7b**, **7d**, **8a-c**) this proton is found at 8.31-8.52 ppm. Both of these effects are also apparent in the benzoates (**6a-d**), but in these cases the signals for both $C_1'H$ and C_6H are superimposed upon the aromatic multiplets.

While the ORD spectra (see Experimental Section) of 5-butyluridine and of the 5-butylcytidines (**8a-c**) exhibit the simple positive Cotton effects expected for pyrimidine β-D-ribonucleosides,[11] the various thionucleosides show more complex spectra. The spectra of 5-butyl-2-thiouridine (**7b**) and 5-butyl-2-thiocytidine (**8a**) show mainly an intense positive Cotton effect centered about 275 mμ with **7b** also exhibiting a smaller negative contribution at 343 mμ. The 4-thio compounds (**6c**, **6d**), however, show much more complex spectra, presumably due in part to $n \rightarrow \pi^*$ transitions of the thiono functions. Similar observations have previously been made for 2-thiouridine,[15] 4-thiouridine,[16,17] 2,4-dithiouridine,[15] and 2-thiocytidine.[14]

The most significant features of the mass spectra of the free 5-butylnucleosides are also recorded in the Experimental Section of this paper. Most of the prominent fragmentations are consistent with previous work on the mass spectra of nucleosides[18] and include well-defined molecular ions, large base + H and base + 2H fragments and frequent base + 30 (base-CH_2O) and base + 41 (base-CHCO?) moieties. In addition, well-defined fragments corresponding to loss of 1-, 2- and 3-carbon fragments from the base + H were apparent in all cases. It is interesting to note that all compounds showed fragments corresponding to (B + H-CH_3) and (B + H-C_2H_5) but that a differentiation occurred between the uridine and cytidine series during loss of the C_3 fragment. Thus, the uridine and 4-thiouridine compounds (**7a-d**) all showed B-41 fragments (presumably B + H-C_3H_6), while the 5-butylcytidines (**8a-c**, **9**) showed instead B-42 fragments (presumably B + H-C_3H_7). Clearly an additional proton transfer from the butyl side chain to the base is involved in the uridine series but a clarification of this point requires further study.

All of the free 5-butylpyrimidine bases and nucleosides reported in this paper have also been examined for possible antiviral activities as reported for 5-butyluridine by Muraoka, *et al.*[1] Preliminary screening against HF strain Herpes Simplex (DNA), IHD strain Vaccinia (DNA), and Type 1, Mahoney strain polio (RNA) viruses was done using a pulp disk diffusion assay with the BGM line of African green monkey kidney cells as host.[19] Under these conditions, none of the compounds showed any significant activity under standard conditions using 30-50 μg of sample per 1-cm diameter disk except that **3** showed some toxicity to the host cells. In addition, the bases (**1**, **2**, **3**) and the nucleosides **6a** and **6b** were examined against both Vaccinia and polio viruses using a plaque reduction assay.§ Under these conditions, **6b** and **2** showed activities similar to those described previously,[1] the nucleoside being more active than the base and inhibiting polio and Vaccinia virus by 39 and 14% at 600 μg/ml. At lower concentrations the activities were insignificant. 5-Butyl-2-thiouracil (**1**) showed moderate activity (71% inhibition) against Vaccinia virus at 300 μg/ml but was cytotoxic to the host cells at higher concentrations. 5-Butyl-2,4-dithiouracil (**3**) was highly cytotoxic at even 10 μg/ml and meaningful antiviral activity could not be demonstrated. On the other hand, 5-butyl-2-thiouridine was essentially noncytotoxic at even 600 μg/ml and under these conditions only inhibited polio and Vaccinia viruses by 16 and 1%, respectively.

It thus appears that the antiviral activity of 5-butyluridine described by Muraoka, *et al.*,[1] is limited to high concentrations ($2 \times 10^{-3} M$, or 600 μg/ml) and that other 5-butylpyrimidine bases and nucleosides show little significant activity against either the DNA or RNA viruses examined.

Experimental Section

General Methods. Thin-layer chromatography (tlc) was conducted using Merck silica gel GF and preparative tlc on 20 × 100 cm glass plates coated with a 1.3-mm layer of Merck silica gel GF. Spots were detected by ultraviolet examination or by spraying with a 5% solution of ammonium molybdate in 10% sulfuric acid followed by brief heating at 150°. Nuclear magnetic resonance spectra were obtained using a Varian HA-100 spectrometer and are reported in ppm downfield of an internal standard of tetramethylsilane. Assignments of sugar protons are, when necessary, confirmed by spin-decoupling studies. Mass spectra were recorded using a CH-4 spectrometer and ORD spectra using a JASCO ORD/UV-5 instrument.

5-Butyl-2-thiouracil (1). A solution of methyl hexanoate (130 g, 1 mole) and ethyl formate (11 g, 1.5 moles) in anhydrous ether (100 ml) was added slowly to an ice-cooled, stirred suspension of sodium (23 g, 1 mole) in ether (225 ml). The mixture was then kept at 4° for 2 days and finally at 23° for 18 hr. The solvent was evaporated, and the residue was dissolved in ethanol (300 ml) containing thiourea (76.1 g, 1 mole) and heated under reflux for 7 hr. After evaporation of the solvent, the residue was dissolved in hot water (250 ml), cooled, and extracted twice with ether (250 ml). The aqueous solution was adjusted to pH 5 with hydrochloric acid, and the resulting precipitate was collected and triturated thoroughly with water. The residue was crystallized from ethanol giving 34.0 g (19%) of **1** with mp 151.5-152.5° (reported[2] mp 151-153°): λ_{max}^{MeOH} 215 (ε 13,100), 276 nm (15,800); mass spectrum (70 eV) *m/e* 184 (M^+), 169 (M − CH_3), 155 (M − C_2H_5), 142 (M − C_3H_6), 141 (M − C_3H_7), 82.

5-Butyluracil (2). A solution of **1** (7.5 g, 41 mmoles) in 10% aqueous monochloroacetic acid (77 ml) was heated under reflux for 6 hr. The mixture was then cooled, and the crystalline product was collected and washed with water. Recrystallization from methanol gave 6.75 g (98%) of **2**, which sublimes above 250° (reported[2] mp 291-293°): λ_{max}^{MeOH} 209 (ε 9500), 265 nm (7700); mass spectrum (70 eV) *m/e* 168 (M^+), 153 (M − CH_3), 151 (M − OH), 139 (M − C_2H_5), 126 (M − C_3H_6), 125 (M − C_3H_7), 82.

5-Butyl-2,4-dithiouracil (3). A mixture of **2** (5 g, 27.1 mmoles) and phosphorus pentasulfide (18.1 g, 81.5 mmoles) was heated under reflux in dioxane (250 ml) for 2 hr at which point tlc using

§The method used is essentially the same as that described by Muraoka, *et al.*,[1b] but using the virus strains and host cells mentioned above.

chloroform–acetone (10:1) showed the reaction to be complete. The solvent was evaporated, and the residue was extracted twice with boiling ether–ethyl acetate (1:1). The extracts were dried and evaporated leaving a residue that was crystallized from ethyl acetate giving 3.92 g (72%) of **3** with mp 232–234°: λ_{max}^{MeOH} 282 (ϵ 24,000), 355 nm (8200); mass spectrum (70 eV) *m/e* 200 (M^+), 185 (M – CH_3), 171 (M – C_2H_5), 167 (M – SH), 158 (M – C_3H_6). *Anal.* Calcd for $C_8H_{12}N_2S_2$ (200.19): C, 47.96; H, 6.04; N, 13.98; S, 32.01. Found: C, 47.45; H, 5.84; N, 13.75; S, 31.79.

5-Butyl-*O*,*S*-bis(trimethylsilyl)-2-thiouracil (4a). A mixture of **1** (5.26 g, 28.6 mmoles) and hexamethyldisilazane (50 ml) was heated under reflux in the presence of ammonium sulfate (100 mg). After 2 hr, a homogeneous solution resulted, and the solvent was evaporated *in vacuo*. The residue was distilled giving 8.38 g (89%) of **4a** with bp 108° (0.1 mm): nmr ($CDCl_3$) 0.36 and 0.47 (s, 9, $SiMe_3$), 0.95 (m, 3, CH_3), 1.4 (m, 4, CH_2CH_2), 2.4 (m, 2, $PyCH_2$), 8.81 (1, s, C$_6$H).

5-Butyl-2,4-bis(trimethylsilyloxy)pyrimidine (4b). A mixture of **2** (5 g, 29.7 mmoles), hexamethyldisilazane (50 ml), and ammonium sulfate (100 mg) was heated under reflux until it became homogeneous (2.5 hr), and then the solvent was evaporated *in vacuo*. Distillation of the residue gave 8.82 g (94%) of **4b** with bp 97–99° (0.03 mm): nmr (CCl_4) 0.34 and 0.46 (s, 9, $SiMe_3$), 1.0 (m, 3, CH_3), 1.45 (m, 4, CH_2CH_2), 2.4 (m, 2, $PyCH_2$), 7.93 (s, 1, C$_6$H); mass spectrum (70 eV) *m/e* 312 (M^+), 297 (M – CH_3), 283 (M – C_2H_5), 270 (M – C_3H_6).

5-Butyl-2,4-bis(trimethylsilylthio)pyrimidine (4c). A mixture of **3** (1 g, 5 mmoles), hexamethyldisilazane (15 ml), and ammonium sulfate (25 mg) was heated under reflux for 4.5 hr and then distilled in a Kugelrohr apparatus[20] giving 0.85 g (50%) of **4c** at bath temperature of 80° and a pressure of 0.05 mm.

2′,3′,5′-Tri-*O*-benzoyl-5-butyluridine (6b). (a) *Via* the Silyl Route. A mixture of mercuric chloride (2.8 g) and mercuric oxide (2.8 g) was ground under benzene and then dried by azeotropic distillation with benzene three times. To the residue in a drybox was added **4b** (8.8 g, 28.2 mmoles) and 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride (**5b**, from 26.8 mmoles of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose and hydrogen chloride in ether) and anhydrous benzene (200 ml). The mixture was heated under reflux for 3 hr at which point tlc using chloroform–ether (10:1) showed the reaction to be complete. Methanol (30 ml) and water (12 ml) were added to the cooled mixture, and the solvents were evaporated *in vacuo*. The residue was extracted several times with hot chloroform, and the extracts were washed with 30% aqueous potassium iodide, then with saturated sodium chloride, and finally with water. The organic phase was dried ($MgSO_4$) and evaporated leaving a foam (17.4 g) that was crystallized twice from ethanol giving 12.24 g (75%) of **6b** with mp 156.5–157.5° (reported[18] mp 157–158°). Chromatography of the mother liquors on a column of silicic acid using chloroform–ether (10:1), followed by crystallization from ethanol, gave a further 1.66 g (total yield 85%) of pure **6b**: λ_{max}^{MeOH} 229 (ϵ 42,000), 265 nm (12,800); *Anal.* Calcd for $C_{34}H_{32}N_2O_9$ (616.64): C, 66.66; H, 5.27; N, 4.57. Found: C, 66.66; H, 5.14; N, 4.48.

(b) *Via* the Mercury Salt. A solution of mercuric chloride (2.04 g, 7.5 mmoles) in ethanol (50 ml) was added to a solution of 5-butyluracil (2.5 g, 14.9 mmoles) and sodium hydroxide (0.60 g, 15 mmoles) in hot water (60 ml). The resulting precipitate was washed with water and dried *in vacuo* giving 3.76 g (82%) of the mercury salt. Separately, 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (6.24 g, 12.4 mmoles) was converted to the 1-chloro derivative by treatment with saturated hydrogen chloride in ether for 2 days at 4° followed by evaporation and coevaporation three times with benzene. The chloro sugar and mercury salt were combined in anhydrous xylene (50 ml) and benzene (25 ml) and heated under reflux for 2 hr. The hot mixture was then filtered, cooled, and precipitated with hexane. The precipitate was dissolved in chloroform, washed with 30% aqueous potassium iodide and then water, dried ($MgSO_4$), and evaporated leaving 7.35 g of a foam. This was chromatographed on a column containing 500 g of silicic acid using chloroform–acetone (20:1). The resulting pure product (4.37 g, 58%) was crystallized from ethanol giving 3.21 g (42%) of **6b** identical with that from (a) above.

5-Butyluridine (7b). A solution of **6b** (2.0 g, 3.26 mmoles) and sodium methoxide (3.26 mmoles) in methanol (25 ml) was heated under reflux for 2 hr. It was then passed through a column containing Dowex 50 (H^+) resin (25 ml), and the resin was washed with aqueous methanol (1:1). The eluates were evaporated to dryness, and the residue was crystallized from 2-propanol giving 0.64 g (65%) of **7b** with mp 161.5–163.5° (reported[18] mp 161–163°): λ_{max}^{MeOH} 267 nm (ϵ 9400); ORD (MeOH) positive Cotton effect $[\Phi]_{309}^{pk}$ 2900°, $[\Phi]_{280}$ 0°, $[\Phi]_{260}^{tr}$ –8700°; mass spectrum (70 eV) *m/e* 300 (M^+) 197 (base + CH_2O), 169 (base + 2H), 168 (base + H), 149 (*m/e* 168 – C_2H_5), 124 (*m/e* 168 – C_3H_6).

2′,3′,5′-Tri-*O*-benzoyl-5-butyl-2-thiouridine (6a). A solution of **4a** (15.89 g, 48.4 mmoles), 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (22.00 g, 43.6 mmoles), and stannic chloride (3.7 ml, 27 mmoles) in anhydrous 1,2-dichloroethane (450 ml) was stirred at room temperature for 16 hr. Tlc using CCl_4–ether (3:1) then showed the essential disappearance of the sugar. The solution was extracted with saturated aqueous sodium bicarbonate and then twice with water, dried, and evaporated leaving a foam (29.5 g) that was crystallized from ethanol giving 14.4 g (53%) of **6a** with mp 122.5–124.5°. Chromatography of the mother liquors on a column of silicic acid (500 g) using CCl_4–ether (4:1), followed by crystallization from ethanol, gave a further 1.13 g (total yield 15.53 g, 57%) of **6a** identical with that above: λ_{max}^{MeOH} 229 (ϵ 53,000), 282 nm (19,500). *Anal.* Calcd for $C_{34}H_{32}N_2O_8S$ (628.71): C, 64.95; H, 5.13; N, 4.46. Found: C, 64.73; H, 5.12; N, 4.46.

5-Butyl-2-thiouridine (7a). A solution of **6a** (2.0 g, 3.18 mmoles) and sodium methoxide (3.18 mmoles) in methanol (15 ml) was heated under reflux for 2 hr. It was then diluted with water and passed through a column containing 25 ml of Dowex 50 (H^+) resin and the resin was washed with 80% methanol. The eluates were evaporated, and the residue was crystallized from aqueous methanol giving 0.87 g (87%) of **7a** with mp 195–197°: λ_{max}^{MeOH} 222 (ϵ 14,400), 278 nm (15,700); ORD (MeOH) multiple Cotton effect $[\Phi]_{343}^{tr}$ –8600°, $[\Phi]_{331}$ 0°, $[\Phi]_{303}^{pk}$ 40,400°, $[\Phi]_{274}$ 0°, $[\Phi]_{244}^{tr}$ –29,300°; mass spectrum (70 eV) *m/e* 316 (M^+), 224 (base + CH=CO), 185 (base + 2H), 184 (base + H), 169 (*m/e* 184 – CH_3), 155 (*m/e* 184 – C_2H_5), 142 (*m/e* 184 – C_3H_6). *Anal.* Calcd for $C_{13}H_{20}N_2O_5S$ (316.38): C, 49.35; H, 6.37; N, 8.86. Found: C, 49.36; H, 6.44; N, 8.76.

5-Butyl-2′,3′,5′-tri-*O*-benzoyl-4-thiouridine (6d). A solution of **6b** (12.24 g, 20 mmoles) and phosphorus pentasulfide (13.30 g, 60 mmoles) in anhydrous dioxane (500 ml) was heated under reflux with stirring for 1.5 hr at which point tlc using CCl_4–ether (3:1) showed the reaction to be complete. After evaporation of the solvent, the residue was extracted twice with hot ethyl acetate and the extracts were washed with water, dried, and evaporated. The residue was crystallized from ethanol giving 10.67 g (85%) of **6d** with mp 171–172°: λ_{max}^{MeOH} 230 (ϵ 38,900), 274 (5100), 282 (4500), 333 nm (16,100); mass spectrum (70 eV) *m/e* 628 (M^+). *Anal.* Calcd for $C_{34}H_{32}N_2O_8S$ (628.61): C, 64.95; H, 5.13; N, 4.46. Found: C, 64.88; H, 5.01; N, 4.38.

5′-Butyl-4-thiouridine (7d). A methanolic solution of **6d** (1.0 g, 1.59 mmoles) was treated with sodium methoxide exactly as for **6a** above. Crystallization of the residue from water gave 0.36 g (72%) of **7d** with mp 167.5–168.5°: λ_{max}^{MeOH} 245 (ϵ 4200), 337 nm (19,300); ORD (MeOH) multiple Cotton effect $[\Phi]_{440}^{pk}$ 800°, $[\Phi]_{390}$ 0°, $[\Phi]_{320}^{tr}$ –1500°, $[\Phi]_{292}$ 0°, $[\Phi]_{284}^{pk}$ 250°, $[\Phi]_{283}$ 0°, $[\Phi]_{252}^{tr}$ –7100°; mass spectrum (70 eV) *m/e* 316 (M^+), 185 (base + 2H), 184 (base + H), 155 (*m/e* 184 – C_2H_5), 151 (*m/e* 184 – SH) 142 (*m/e* 184 – C_3H_6). *Anal.* Calcd for $C_{13}H_{20}N_2O_5S$ (316.38): C, 49.35; H, 6.37; N, 8.86. Found: C, 49.41; H, 6.39; N, 8.78.

2′,3′,5′-Tri-*O*-benzoyl-5-butyl-2,4-dithiouridine (6c). A mixture of **6a** (1.26 g, 2 mmoles) and phosphorus pentasulfide (1.33 g, 6 mmoles) in anhydrous dioxane (50 ml) was heated under reflux with stirring for 1.5 hr. The mixture was filtered, and the filtrate was evaporated to dryness. The residue was dissolved in ethyl acetate, washed twice with water, dried, and evaporated leaving a yellow foam. Crystallization from methanol gave 1.10 g (85%) of **6c** with mp 97–99°: λ_{max}^{MeOH} 230 (ϵ 40,700), 282 (25,600), 345nm (sh, 11,600); mass spectrum (70 eV) *m/e* 445 (M – base), 322, 200 (base + H). *Anal.* Calcd for $C_{34}H_{32}N_2O_7S_2$ (644.77): C, 63.34; H, 5.00; N, 4.35. Found: C, 63.15; H, 5.06; N, 4.25.

5-Butyl-2,4-dithiouridine (7c). A solution of **6c** (1.1 g, 1.7 mmoles) was treated under reflux with sodium methoxide (1.8 mmoles) in methanol (15 ml) for 2 hr. The solution was then neutralized with Dowex 50 (H^+) resin, filtered, and evaporated to dryness. The residue was triturated with chloroform, and the insoluble residue was crystallized from water giving 0.405 g (72%) of **7c** with mp 156.5–157.5°: λ_{max}^{MeOH} 281 (ϵ 25,100), 353 nm (11,700); ORD (MeOH) multiple Cotton effect $[\Phi]_{469}^{pk}$ 710°, $[\Phi]_{430}$ 0°, $[\Phi]_{374}^{tr}$ –7000°, $[\Phi]_{351}$ 0°, $[\Phi]_{294}^{pk}$ 17,100°, $[\Phi]_{279}$ 0°, $[\Phi]_{260}^{tr}$ –19,700°; mass spectrum (70 eV) *m/e* 332 (M^+), 240 (base + CHCO), 201 (base + 2H), 200 (base + H), 185 (*m/e* 200 – CH_3), 171 (*m/e* 200 – C_2H_5), 167 (*m/e* 200 – SH), 158 (*m/e* 200 – C_3H_6). *Anal.* Calcd for

$C_{13}H_{20}N_2O_4S_2$ (332.45): C, 46.97; H, 6.06; N, 8.43. Found: C, 46.55; H, 6.06; N, 8.32.

5-Butylcytidine (8a). A solution of **6d** (1.26 g, 2 mmoles) in saturated methanolic ammonia (15 ml) was heated in a stainless steel bomb for 24 hr at 100°. The solvent was then evaporated, and the residue was partitioned between water and ether. The aqueous phase was passed through a column containing 25 ml of Dowex 50 (H^+) resin and the resin was washed thoroughly with water. Elution with 2 *N* ammonium hydroxide gave 0.6 g of a residue that was not readily crystallized as either the free base or the hydrochloride and could be shown by tlc using chloroform–methanol (4:1) to contain a less polar impurity. The material was dissolved in methanol–water (3:7) and applied to a 2 × 30 cm column of Bio-Rad AG-1 × 2 (OH^-) resin. Elution with methanol–water (3:7) gave a small peak closely followed by a large one. Evaporation of the major peak (10,000 optical density units at 280 nm) gave 416 mg (70%) of a chromatographically homogeneous syrup that could not be crystallized. Addition of ether to its solution in methanol gave 360 mg (60%) of 8a as a solid with mp 80–82°: $\lambda_{max}^{H_2O}$ 213 (ε 13,000), 278 nm (8000); ORD (H_2O) positive Cotton effect $[\Phi]_{293}^{pk}$ 5000°, $[\Phi]_{279}$ 0°, $[\Phi]_{238}$ −9800°; mass spectrum (70 eV) *m/e* 299 (M^+), 207 (base + CHCO), 196 (base + CH_2O), 168 (base + 2H), 167 (base + H), 152 (*m/e* 167 − CH_3), 138 (*m/e* 167 − C_2H_5), 124 (*m/e* 167 − C_3H_7). *Anal.* Calcd for $C_{13}H_{21}N_3O_5$ (299.33): C, 52.16; H, 7.07; N, 14.04. Found: C, 52.01; H, 7.36; N, 13.69.

Evaporation of the minor peak gave 49 mg (8%) of 5-butyl-N^4-methylcytidine identical in every way with the material described below.

5-Butyl-N^4-methylcytidine (8b). A solution of **6d** (2.51 g, 4 mmoles) and methylamine (3.2 g) in methanol (25 ml) was heated in a stainless steel bomb for 16 hr at 100°. Following evaporation of the solvent, the residue was partitioned between water and ether and the aqueous phase was absorbed on a column containing 50 ml of Dowex 50 (H^+) resin. The resin was washed with water (1.5 l.) and then eluted with 1 *N* ammonium hydroxide. The eluate was evaporated leaving 1.23 g of a residue that could not be obtained in obviously crystalline form. The substance was precipitated with ether from methanol giving 1.17 g (93%) of 8b as a white solid with an indefinite mp at 106–114°: $\lambda_{max}^{H_2O}$ 217 (ε 10,000), 234 sh (7900), 278 nm (8600); ORD (MeOH) positive Cotton effect $[\Phi]_{298}^{pk}$ 5900°, $[\Phi]_{282}$ 0°, $[\Phi]_{244}^{tr}$ −13,100°. *Anal.* Calcd for $C_{14}H_{23}N_3O_5$ (313.36): C, 53.66; H, 7.40; N, 13.41. Found: C, 53.64; H, 7.69; N, 13.02.

5-Butyl-N^4-dimethylcytidine (8c). A solution of **6d** (2.71 g, 4.3 mmoles) and dimethylamine (10.5 g) in methanol (30 ml) was heated in a stainless steel bomb at 100° for 16 hr. After evaporation of the solvent, the residue was partitioned between water and ether. Partial evaporation of the water led to crystallization of 0.35 g (28%) of 5-butyl-4-thiouridine with mp 166–167°. The mother liquors were absorbed on Dowex-50 (H^+) resin, and the resin was thoroughly washed with water and then eluted with 1 *N* ammonium hydroxide. The eluate was evaporated, and the residue was precipitated with ether from methanol giving 475 mg (36%) of 8c as a homogeneous white monohydrate which could not be obtained in an obviously crystalline from: λ_{max}^{MeOH} 207 (ε 19,300), 288 nm (11,200); ORD (MeOH) positive Cotton effect $[\Phi]_{306}^{pk}$ 4100°, $[\Phi]_{289}$ 0°, $[\Phi]_{254}^{tr}$ −8000°. *Anal.* Calcd for $C_{15}H_{25}N_3O_5 \cdot H_2O$ (335.40): C, 52.16; H, 7.88; N, 12.17. Found: C, 52.64; H, 7.46; N, 12.16.

5-Butyl-2-thiocytidine (9). A mixture of **6a** (1.5 g, 2.38 mmoles) and phosphorus pentasulfide (1.59 g, 7.14 mmoles) in anhydrous dioxane (50 ml) was heated under reflux for 1.5 hr at which point tlc using CCl_4–ether (3:1) showed conversion to **6c** to be complete. The mixture was filtered, and the filtrate was evaporated and partitioned between water and ethyl acetate. The organic phase was dried ($MgSO_4$) and evaporated leaving 1.60 g of crude **6c**. This was directly dissolved in methanol (20 ml), saturated with ammonia, and heated at 100° for 16 hr. Following evaporation of the solvent, the residue was partitioned between water and ether and the aqueous phase was applied to a 2.5 × 35 cm column of Bio-Rad AG-1-X2 (OH^-) resin. Elution of the column with 2 l. of methanol–water (3:7), followed by a gradient of 30–100% methanol, gave several small peaks followed by one large peak at roughly 75% methanol. Evaporation of this pooled peak, followed by precipitation with ether from methanol, gave 320 mg (44%) of 9 contaminated with a trace of more polar material. This was removed by preparative tlc using chloroform–methanol (4:1) followed by precipitation with ether from methanol giving 267 mg (35%) of 9 which melted at 106–109°; λ_{max}^{MeOH} 258 nm (ε 20,800); $\lambda_{max}^{pH\ 1}$ 278, 232 nm; ORD (MeOH) positive Cotton effect $[\Phi]_{290}^{pk}$ 13,400°, $[\Phi]_{276}$ 0°, $[\Phi]_{254}^{tr}$ −32,900°, $[\Phi]_{230}^{pk}$ −2100°; mass spectrum (70 eV) *m/e* 315 (M^+), 297 (M − H_2O), 279 (M − $2H_2O$), 184 (base + 2H), 183 (base + H), 168 (*m/e* 183 − CH_3), 154 (*m/e* 183 − C_2H_5), 140 (*m/e* 183 − C_3H_7). *Anal.* Calcd for $C_{13}H_{21}N_3O_4S \cdot H_2O$ (333.34): C, 46.84; H, 6.96; N, 12.61. Found: C, 47.57; H, 6.60; N, 12.47.

1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-5-butyl-4-ethoxy-2(1*H*)-pyrimidinone (10). A solution of **6b** (1.23 g, 2 mmoles), thionyl chloride (1.6 ml, 20 mmoles), and DMF (0.1 ml) in alcohol-free chloroform# (10 ml) was heated under reflux for 16 hr. The solvent was then evaporated leaving 1.35 g of a dry residue. This material was purified by preparative tlc using chloroform–ether (10:1) and the major uv-absorbing band was eluted with acetone and evaporated leaving 0.52 g (41%) of **10** as a dry foam: λ_{max}^{MeOH} 228 (ε 45,800), 276 (8100), 282 nm (8100). *Anal.* Calcd for $C_{36}H_{36}N_2O_9$ (640.70): C, 67.49; H, 5.66; N, 4.37. Found: C, 67.95; H, 5.59; N, 4.32.

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#Prepared by passing anhydrous chloroform through a column of activated alumina.