[Chem. Pharm. Bull. 23(11)2614—2619(1975)]

UDC 547.455'854.4.057 : 547.569.1.04 : 541.459

Nucleosides and Nucleotides. XIV. Synthesis of 6-Alkylthiouridines and Uridine-6-sulfonic Acid*,[1]

Hideo Inoue, Setsuko Tomita and Tohru Ueda

Faculty of Pharmaceutical Sciences, Hokkaido University[2]

(Received May 19, 1975)

Treatment of 5′-*O*-acetyl-2′,3′-*O*-isopropylidene-5-bromouridine with benzyl mercaptan yielded both 5-benzylthio and 6-benzylthio derivatives, (IIa) and (IIIa). Deprotection of those derivatives gave 5-benzylthiouridine (IIc) and 6-benzylthiouridine (IIIc). Treatment of IIIa with methoxide followed by H_2S afforded 2′,3′-*O*-isopropylidene-6-thiouridine (IV). Methylation of IV gave 6-methylthiouridine (VI) after deblocking. Oxidation of IV with hydrogen peroxide afforded uridine-6-sulfonic acid (VII), an orotidine analog. Nuclear magnetic resonance and circular dichroism data were given and conformations of these nucleosides were discussed.

Recent reports from our laboratory disclosed that the treatment of 5-bromo-uridines and -cytidines with sodium cyanide under mild reaction conditions gave their 6-cyano derivatives which, in turn, were converted to the respective 6-carboxylic acids (orotidine and its cytidine counterpart).[3,4] The reaction involved the initial nucleophilic attack of cyanide ion to the C-6 position of the pyrimidines to give the 5-bromo-6-cyano-5,6-dihydro intermediate, which on dehydrobromination affords 6-cyanopyrimidines. Similar reactions affording 6-substituted pyrimidine nucleosides have been advanced in the formation of the O^6,5′-cyclo-uridine[5] and -cytidine[6] derivatives by the treatment of 5-halogenopyrimidine nucleosides with alkoxides in which the nucleophilic attack occurred intramolecularly. We have also observed the similar reaction yielding 6,5′-*S*(and *N*)-cyclouridines starting from a 5′-thio- and 5′-amino-derivatives of 5-bromouridine.[7] Bardos and co-workers have recently reported[8] the reaction of 1-methyl-5-bromouracil with sodium hydrogen sulfide to give the 5-mercapto derivative together with an appreciable amount of dehalogenated product, 1-methyluracil. Hayatsu and co-workers have also observed similar results in a reaction of 5-bromo-2′-deoxyuridine with cysteine.[9]

Since we have observed the intramolecular attack of the sulfur nucleophile as well as the intermolecular attack of cyanide ion with 5-bromouridines, the formation of the 6-mercapto derivative by the intermolecular addition-elimination path in 5-halogenopyrimidine nucleosides may well be expected, although no formation of such derivatives were observed in the preceding cases.[8,9]

The present report describes the formation of 6-benzylthiouridine derivatives and its conversion to 6-thiouridine, 6-methylthiouridine and uridine-6-sulfonic acid, potential orotidylate decarboxylase inhibitors in pyrimidine nucleotide biosynthesis.

* Dedicated to the memory of Prof. Eiji Ochiai.

1) Part XIII: K. Miura and T. Ueda, *Chem. Pharm. Bull.* (Tokyo), **23**, 2064 (1975).
2) Location: *Kita-12, Nishi-6, Kita-ku, Sapporo, 060, Japan.*
3) H. Inoue and T. Ueda, *Chem. Pharm. Bull.* (Tokyo), **19**, 1743 (1971).
4) T. Ueda, H. Inoue and A. Matsuda, *Ann. N.Y. Acad. Sci.*, **255**, 121 (1975).
5) B.A. Otter, E.A. Falco, and J.J. Fox, *J. Org. Chem.*, **34**, 1390 (1969).
6) D. Lipkin, C. Cori, and M. Sano, *Tetrahedron Letters*, **1968**, 5993.
7) H. Inoue and T. Ueda, Abstr. Papers 93 rd Annual Meeting Pharm. Soc. Japan, Tokyo, April 1973, Part II, p. 117.
8) L. Szabo, T.I. Kalman, and T.J. Bardos, *J. Org. Chem.*, **35**, 1434 (1970).
9) Y. Wataya, K. Negishi, and H. Hayatsu, *Biochemistry*, **12**, 3992 (1973).

We selected 5′-*O*-acetyl-2′,3′-*O*-isopropylidene-5-bromouridine[3] (I) as a starting material, since the protection of the sugar hydroxylic function seemed to be necessary for setting up the homogeneous reaction and avoiding the possible side reaction, the cyclonucleoside formation. Furthermore the removal of the acetyl or the isopropylidene group would facilitate the derivatization of newly synthesized 6(5)-substituted uridines at the 5′- or 3′(2′)-hydroxyl selectively.

Compound I was treated with an excess of benzyl mercaptan in the presence of pyridine at 120° for 21 hours in a sealed tube. A prior survey of the products in the reaction mixture on thin-layer chromatography (TLC) (silica gel, $CHCl_3$–EtOH, 10: 1) showed that the reaction proceeded almost completely giving a new spot (*Rf* 0.58) and starting material (*Rf* 0.52) or the dehalogenated compound (*Rf* 0.46) were found to be practically negligible. From the eluate through silicic acid column chromatography a crystalline compound was separated which turned out to be 5′-*O*-acetyl-2′,3′-*O*-isopropylidene-5-benzylthiouridine (IIa). The structure of IIa was confirmed by spectral analyses. The ultraviolet (UV) spectra of IIa was closely similar to those of 5-alkylthiouracils,[10] showing a characteristic plateau at a main absorption region. Nuclear magnetic resonance (NMR) spectra showed C-6 proton and phenyl protons at 7.20 ppm with no signal corresponding to that of C-5 proton around 6 ppm.

Compound IIa was next subjected to deacetylation by the methoxide treatment to give 2′,3′-*O*-isopropylidene-5-benzylthiouridine (IIb) as a non-crystallizable form, which was subsequently treated with 50% aqueous formic acid to afford crystalline 5-benzylthiouridine (IIc) in a yield of 71% from IIa. The spectral characteristics of IIc will be discussed later. The mother liquor from which IIa was isolated was treated with methoxide in methanol and new crystals found out to be 2′,3′-*O*-isopropylidene-6-benzylthiouridine (IIIb) were obtained in 34% yield from I as a mono-ethanolate. The NMR spectra of IIIb showed a signal at 5.64 ppm indicative of that of C-5 proton, together with the benzyl protons at 7.33 and 4.13 ppm. Deacetonation of IIIb in 50% aqueous formic acid gave the crystalline 6-benzylthiouridine (IIIc) in 74% yield.

Several attempts to obtain either one of IIIa or IIa as the predominat product in the reaction of I with benzyl mercaptan, such as changing the ratios of the reactants, temperature, reaction time, met with little success. The use of other mercaptans, namely ethyl mercaptan, did not give the satisfactory results under similar reaction conditions. This is presumably because of the inferior nucleophilic character of the ethyl mercaptan or alkyl mercaptans than that of benzyl mercaptan.

Therefore another approach was deviced for the general method of preparation of 6-alkylthiouridines. Treatment of IIIb with triethylamine-dimethylformamide, previously saturated with hydrogen sulfide, at 60° for 16 hours afforded a single product, 2′,3′-*O*-isopropylidene-6-thiouridine (IV). From the UV spectral measurements it is indicative that IV dissociates in the neutral medium (see experimental part). The nucleophilic substitution of the benzylthio group with sulfhydryl ion must have taken place rather readily at the 6-position as in the case of 4-methylthio group in certain pyrimidin-2-one.[11] Without further purification of the product (IV) it was converted to the sodium salt and treated with methyl iodide in acetone under neutral condition to avoid over-methylation at N-3 of IV. Methylation proceeded quantitatively within 30 minutes. After removal of the salts by column chromatography 2′,3′-*O*-isopropylidene-6-methylthiouridine (V) was isolated as a glass from IIIb in high yield. Subsequent treatment of V with 50% aqueous formic acid afforded 6-methylthiouridine (VI) in 86% yield. The above sulfhydrolytic procedure may be applicable to the synthesis of various 6-alkylthiouridines in general. Winkley and Robins had reported[12] the synthesis of 6-methylthio-3-(β-D-ribofuranosyl)pyrimidine-2,4-dione (a N-3 ribosyl isomer of VI) by

10) M.P. Kotick, C. Szantay, and T.J. Brados, *J. Org. Chem.*, **34**, 3806 (1969).
11) T. Ueda, Y. Iida, K. Ikeda, and Y. Mizuno, *Chem. Pharm. Bull.* (Tokyo), **16**, 1788 (1968).
12) M.W. Winkley and R.K. Robins, *J. Chem. Soc.*(*C*), **1969**, 791.

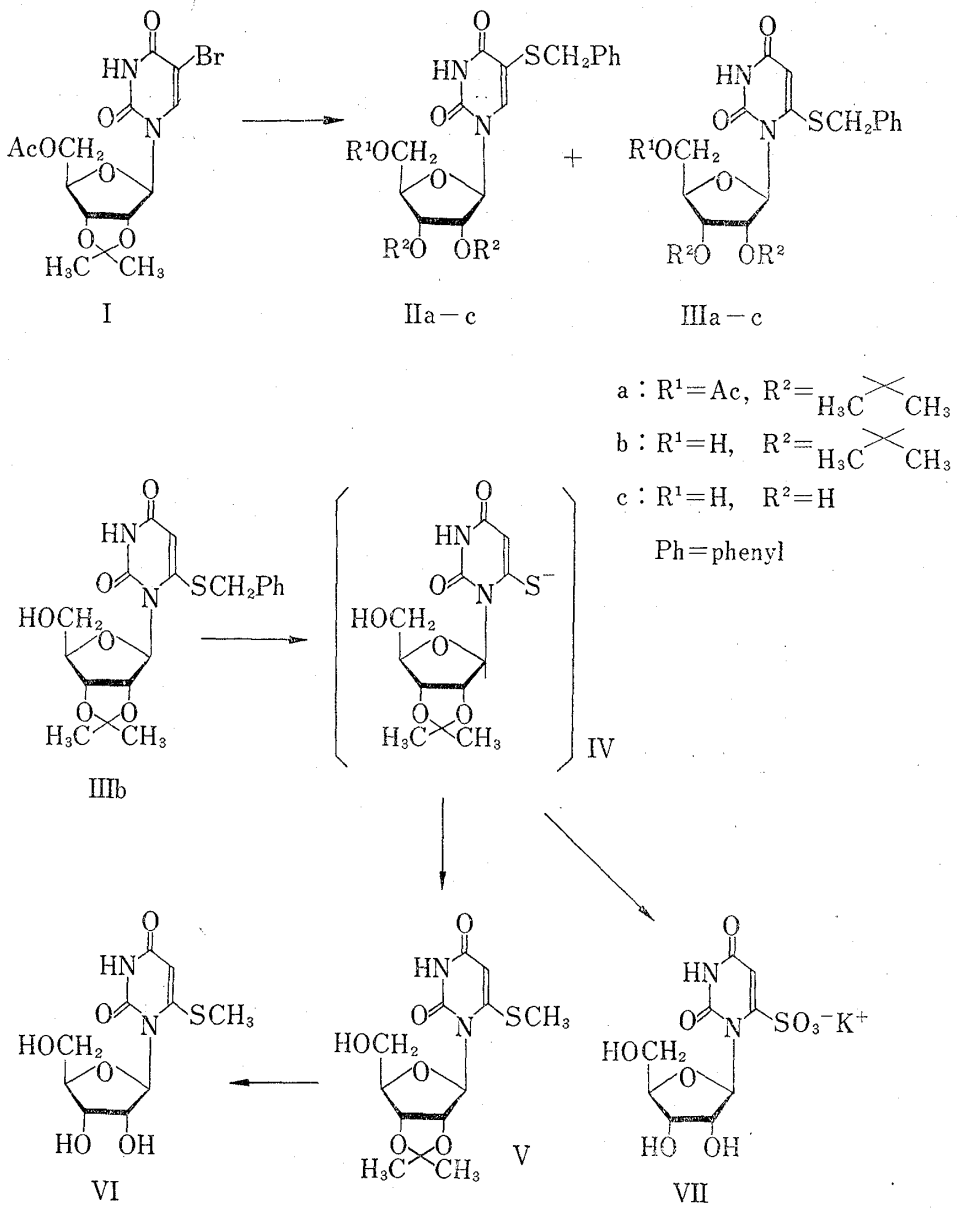

TABLE I. NMR Chemical Shifts (δ) of 5(6)-Substituted Uridines taken at 60 MHz in d_6-DMSO-D_2O[a]

Substituted uridines	5-H and/or 6-H	1′-H ($J_{1',2'}$, Hz)	2′-H ($J_{2',3'}$, Hz)	3′-H ($J_{3',4'}$, Hz)	4′-H and 5′-H	Other protons
6-Benzylthio-(IIIc)	5.61, s	5.71, d (3.8)	4.58, dd (6.0)	4.08, pt (6.0)	3.9—3.2	$-SCH_2-$ 4.32, s −phenyl 7.37
6-Methylthio-(VI)	5.48, s	5.71, d (3.8)	4.61, dd (6.0)	4.07, pt (6.0)	3.9—3.3	$-SCH_3$ 2.50, s
6-Sulfonate (VII)	6.07, s	6.39, d (2.6)	4.42, dd (6.0)	4.16, pt (6.0)	3.9—3.3	
5-Benzylthio-(IIc)	7.91, s	5.71, d (3.0)	—[b]	—[b]	4.1—3.3	$-SCH_2-$ 3.93, s −phenyl 7.23
Uridine	5.63, d 7.86, d	5.76, d (4.5)	—[b]	—[b]	4.2—3.4	

a) The concentrations of the samples were about 10% in 10%–D_2O in d_6-DMSO.
b) Signals were involved in 4′- and 5′-H region.

the ribosylation of a silylated 6-methylthiouracil. In this instance no formation of the 1-ribosyl derivative, VI, was observed.

In recent years we have been involved in the synthesis of orotidine and its analogs including uridine-6-acetic acid, starting from naturally occurring pyrimidine nucleosides. Uracil-6-sulfonic acid had been synthesized[13] and tested as an inhibitor of orotidylate decarboxylase and pyrophosphorylase.[14] As more effective analog we carried out the synthesis of this nucleoside, uridine-6-sulfonic acid. Potassium salt of IV was treated with hydrogen peroxide in aqueous formic acid at room temperature for 3 hours. Oxidation of the 6-thio group and simultaneous deacetonation proceeded to afford potassium salt of uridine-6-sulfonic acid (VII) in 84% yield. The compound migrates equally with orotidine on a paper electrophoretic run.

Recently we have found that the sulfhydrolysis of cytidines and certain activated adenosines afforded their respective 4-thio and 6-thio derivatives.[1,15,16] 6-Aminouracils were also converted to 6-thiouracils by the similar manner.[17] This procedure will constitute an alternate route for the introduction of the 6-thio group in pyrimidine nucleosides and studies along this line are presently undertaken.

In terms of the conformational studies of nucleosides the spectroscopic comparisons with 5- and 6-substituted uridines seem to be interesting. Compound IIIc, VI, and VII having bulky group at C-6 must possess a *syn*-conformation in terms of the base orientations. The NMR chemical shifts and coupling constants of protons at the sugar portion were given in Table I. As have been documented by Schweizer and co-workers[18] the chemical shifts of 2′- and 3′-H of 6-benzylthiouridine (IIIc) were deshielded as compared with those of 5-benzylthio derivative (IIc), although its proton signals were buried in the 4′- and 5′-H region. This is in accord with the assumption[18] that the 2-carbonyl group of IIIc should be located over the furanose ring rather than orientating outside of the ring (*anti*-conformation). The same situation holds in the case of VI as well as VII. The comparisons of the coupling constants between 1′,2′ and 3′,4′ protons in IIIc, VI, and VII would show that the sugar puckering equilibrium in these 6-thio derivatives is in slightly predominant 3′-endo mode (N-type).[19]

The alternate tool for the analysis of nucleoside conformations is the circular dichroism (CD) spectra. Measurement of the CD spectra of IIIc and VI exhibits a positive Cotton band at the longer wavelength absorption region. All 6-substituted pyrimidine nucleosides reported,[4,20] except 6-methyl-uracil and -cytosine nucleosides,[21] showed similar Cotton bands. This would mean that the rule for the sign of longer wave-length CD band and base-sugar conformations in pyrimidine nucleosides (*anti*=positive, *syn*=negative)[22] seems to be an oversimplification. Further studies including solvents and temperature dependencies as well as the theoretical considerations are necessary to establish more reliable rule. It is to be noted that the CD spectra of VII showed a weak negative Cotton band around 288 nm region which is far out of the main absorption of the chromophore (264 nm), where the positive Cotton band is observable (258 nm, +3200).

13) S.B. Greenbaum and W.L. Holmes, *J. Am. Chem. Soc.*, **76**, 2899 (1954).
14) W.L. Holmes, *J. Biol. Chem.*, **223**, 677 (1956).
15) T. Ueda, K. Miura, M. Imazawa, R. Iwata, and K. Odajima, *Tetrahedron Letters*, **1971**, 2507.
16) T. Ueda, K. Miura, M. Imazawa, and K. Odajima, *Chem. Pharm. Bull.* (Tokyo), **22**, 2377 (1974).
17) H. Ogawa, K. Miura, and T. Ueda, Abstr. Paper s, 93 rd Annual Meeting Pharm. Soc. Japan, Tokyo, April **1973**, Part II, p. 132.
18) M.P. Schweizer, E.B. Banta, J.T. Witkowski, and R.K. Robins, *J. Am. Chem. Soc.*, **95**, 3770 (1973).
19) C. Altona and M. Sundaralingam, *J. Am. Chem. Soc.*, **95**, 2333 (1973).
20) A. Holý, *Collection Czech. Chem. Commun.*, **37**, 1555 (1972).
21) D.W. Miles, M.J. Robins, R.K. Robins, M.W. Winkley, and H. Eyring, *J. Am. Chem. Soc.*, **91**, 824, 831 (1969).
22) T.L.V. Ulbricht, "Synthetic Procedures in Nucleic Acid Chemistry," Vol. 2, W.W. Zorbach and R.S. Tipson, Eds. Wiley-Interscience. New York, N.Y., 1973, pp. 177—213.

Experimental

The NMR spectra were recorded at 35° on a Hitachi H-60 spectrometer using tetramethylsilane as internal standard. UV spectra were measured on a Hitachi 3T spectrophotometer. CD spectra were recorded on a JASCO J-40 recording spectropolarimeter in aqueous solution with 8 times accumulations.

5′-*O*-Acetyl-2′,3′-*O*-isopropylidene-5-benzylthiouridine (IIa)——To a solution of 4.0 g (9.9 mmoles) of I in 10 ml of pyridine was added 20 ml of benzyl mercaptan and the mixture was heated at 120° for 21 hr in a stainless steel container. The reaction mixture was taken up in 200 ml of EtOAc and the solution was washed with 100 ml of H_2O. Washing was repeated three times with 50 ml of H_2O and the organic layer was dried over sodium sulfate. After removal of the solvent under reduced pressure the residual oil was diluted with $CHCl_3$ and applied to a column of silicic acid (150 g, 4.6×17 cm). At first benzyl mercaptan was eluted with $CHCl_3$. From the eluates with 4% EtOH–$CHCl_3$ a mixture of IIa and IIIa was obtained. IIa was crystallized from EtOH to yield 1.77 g (40%), mp 155—156.5°. A portion was recrystallized from EtOH to give an analytical sample, mp 156.5—157.5°, as pale green prisms, *Anal.* Calcd. for $C_{21}H_{24}O_7N_2S$: C, 56.24; H, 5.39; N, 6.25; S, 7.15. Found: C, 56.43; H, 5.39; N, 6.29; S, 7.05. UV λ_{max}^{EtOH} nm, 251, 295 sh; λ_{mi}^{EtOH} nm, 240. NMR ($CDCl_3$),: 1.34, 1.55 (3H, each, isop-CH_3), 2.08 (3H, Ac), 3.92 (2H, –SCH_2–), 4.23 (3H, 4′-H, 5′-H), 4.60 (2H, 2′-H, 3′-H), 5.70 (1H, s, 1′-H), 7.20 (6H, 6-H, phenyl), 9.56 (1H, bs, N^3–H). Mass Spectrum *m/e*: 448 (M^+).

2′,3′-*O*-Isopropylidene-6-benzylthiouridine (IIIb)——The mother liquor from which IIa was mostly removed, described in the preceding section, was evaporated to dryness and the residue was dissolved in 40 ml of abs. MeOH to which was added 4 ml of 2N $NaOCH_3$ in MeOH. After one hr the solution was neutralized with the addition of Dowex 50 W (H^+ form) resin. The mixture was filtered and the resin was washed with MeOH. The combined filtrates were evaporated and the residual gum was dissolved in hot EtOH–hexane. On cooling, colorless needles (IIIb, 1.52 g, 34% from I) were separated, mp 139—141°. One recrystallization from EtOH–hexane gave an analytical sample, mp 141—143°. *Anal.* Calcd. for $C_{19}H_{22}O_6N_2S\cdot C_2H_5OH$: C, 55.74; H, 6.24; N, 6.19; S, 7.08. Found: C, 55.64; H, 6.14; N, 6.24; S, 7.11. UV λ_{max}^{EtOH} 278 nm; λ_{min}^{EtOH} 247 nm. NMR ($CDCl_3$): 1.33, 1.53 (3H, each, isop-CH_3), 3.81 (2H, bd, 5′-H), 4.13 (3H, m, –SCH_2– and 4′-H), 4.99 (1H, dd, 3′-H), 5.25 (1H, dd, 2′-H), 5.64 (1H, s, 5-H), 6.06 (1H, d, 1′-H), 7.33 (5H, s, phenyl), 9.96 (1H, bs, N^3-H). Mass Spectrum *m/e*: 391 $(M-CH_3)^+$.

5-Benzylthiouridine (IIc)——To a solution of 3.0 g (6.7 mmoles) of IIa in 50 ml of abs. MeOH was added 5 ml of 2N $NaOCH_3$ in MeOH. The solution was neutralized after 4 hr with the addition of Dowex 50 W (H^+ form) resin which was filtered and washed with MeOH. The combined filtrates were evaporated to dryness. The residual glass (IIb) was taken up in 150 ml of 50% aqueous HCO_2H and kept for overnight at room temperature. The solution was concentrated and after repeated evaporation with the addition of EtOH, the residual glass was checked on TLC (silica gel, $CHCl_3$–EtOH, 5: 1), which showed minor spots having higher *Rf* values than that of major spot of IIc. These were presumably the formylated derivatives of IIc. The residue was dissolved in 40 ml of abs. MeOH to which was added 4 ml of 2N $NaOCH_3$ in MeOH and kept for 1 hr followed by neutralization with the addition of Dowex 50 W (H^+ form) resin. After evaporation of the filtrates the residue was crystallized from EtOH to give 1.75 g, 71%, of IIc, mp 173—174°. *Anal.* Calcd. for $C_{16}H_{18}O_6N_2S$: C, 52.45; H, 4.95; N, 7.65; S, 8.75. Found: C, 52.27; H, 4.98; N, 7.56; S, 8.58. UV $\lambda_{max}^{H_2O}$ nm (ε): 268.5 (6900); $\lambda_{min}^{H_2O}$ nm (ε): 250 (6200); $\lambda_{plateau}^{1NNaOH}$ nm (ε): 260—280 (5400 at 270). CD spectrum nm (θ): peak 282 (+11900), 264 (0), sh 250 (−16200), trough 226 (−71400). NMR: see Table I.

6-Benzylthiouridine (IIIc)——A solution of IIIb (1.0 g, 2.2 mmoles as a mono-ethanolate) in 50 ml of 50% aqueous HCO_2H was kept at room temperature overnight. After concentration of the solvent and repeated evaporation with the addition of EtOH, the residual glass was dissolved in 20 ml of MeOH to which was added 1.5 ml of 2N $NaOCH_3$ in MeOH and kept for 1 hr. The solution was neutralized by the addition of Dowex 50 W (H^+ form) resin, which was filtered and washed with MeOH. The combined fitrates were evaporated and the residue was crystallized from EtOH to give 0.6 g, 74%, of IIIc as fine needles, mp 169—170° (resolidified at 172°, decomp at 225°). *Anal.* Calcd. for $C_{16}H_{18}O_6N_2S$: C, 52.45; H, 4.95; N, 7.65; S, 8.75. Found: C, 52.13; H, 4.89; N, 7.54; S, 8.70. UV $\lambda_{max}^{H_2O}$ nm (ε): 283 (13300), $\lambda_{min}^{H_2O}$ nm (ε): 250.5 (4300), λ_{max}^{1NNaOH} nm (ε): 282.5 (10400), λ_{min}^{1NNaOH} nm (ε): 255 (5200). CD spectra nm (θ): trough 312 (−600), 304 (0), peak 283.5 (+3900), 265 (0), infl. 243 (−3100). NMR: see Table I.

6-Methylthiouridine (VI)——Compound IIIb (3.0 g, 6.6 mmoles as a mono-ethanolate) was placed in an ice-cooled stainless steel tube to which was added a solution of 3 ml of triethylamine and 60 ml of dimethylformamide saturated with H_2S under ice cooling, and sealed. The tube was kept at 60° for 16 hr. After vaporiazation of most of H_2S by streaming N_2 gas the reaction mixture was concentrated. The residual syrup was taken up in 100 ml of H_2O, washed twice with $CHCl_3$ to remove resulting benzyl mercaptan. Paper chromatography of the aqueous layer showed a single spot (*Rf* 0.54; EtOH–1M NH_4OAc, pH 7, 5: 2). The UV spectra of the product were as follows: $\lambda_{max}^{H_2O}$ 311 and 233 nm, $\lambda_{min}^{H_2O}$ 260 nm; $\lambda_{max}^{0.5N\ HCl}$ 314 nm; $\lambda_{min}^{0.5N\ HCl}$ 263 nm; $\lambda_{max}^{0.5N\ NaOH}$ 302 nm, $\lambda_{min}^{0.5N\ NaOH}$ 258 nm. These spectra are indicative of 6-thiouridine structure (IV). Without further attempt to isolate IV, the aqueous solution was made alkaline by the addition of 7.4 ml of 1N NaOH. After evaporation of H_2O the residue was dissolved in 50 ml of acetone and added one drop of acetic acid. CH_3I (0.5 ml, 8 mmoles) was added to the solution and after 30 min at room temperature the

solvent was removed to leave a mass. This was taken up in $CHCl_3$ and applied on a column of silica gel (65 g, 3.1 × 16 cm) and elution was performed with $CHCl_3$ and 5% EtOH–$CHCl_3$. The product (V) was eluted from the latter fraction which was concentrated to leave a non-crystallizable glass, 2.2 g. Compound V was taken up in 100 ml of 50% aqueous HCO_2H and the solution was allowed to stand overnight at room temperature. After evaporation of the solvents and repeated evaporation with the addition of EtOH the residue was dissolved in 50 ml of abs. MeOH containing 2 ml of 2N $NaOCH_3$ and left for 1 hr. The solution was neutralized by the addition of Dowex 50 W (H^+ form) resin. The resin was filtered and washed with MeOH. The filtrates and washings were evaporated and the residue was taken up in EtOH. On concentration of the solvent cream-colored crystals of VI, 1.64 g, 86%, mp 159—161.5°, were separated. One recrystallization from MeOH afforded a pure sample, mp 163—165.5° (resolidified at 166°, decomp. at 230°). *Anal.* Calcd. for $C_{10}H_{14}O_6N_2S$: C, 41.38; H, 4.86; N, 9.65; S, 11.05. Found: C, 41.11; H, 4.83; N, 9.64; S, 10.81. UV $\lambda_{max}^{H_2O}$ nm (ε): 281 (15400), $\lambda_{min}^{H_2O}$ nm (ε): 248.5 (3100), $\lambda_{max}^{1N\ NaOH}$ nm (ε): 280 (11900), $\lambda_{min}^{1N\ NaOH}$ nm (ε): 253 (4200). CD spectra nm (θ): peak 283 (+8800), 256 (0), trough 228.5 (−7500), peak 217.5 (−3900). NMR: see Table I.

Potassium Uridine-6-sulfonate (VII)——To an aqueous solution of IV prepared from IIIb (1.0 g, 2.2 mmoles as a mono-ethanolate) as described in the previous section, was added 0.15 g (2.3 mmoles) of KOH. The solution was concentrated and the residual syrup was dissolved in 50 ml of 88% aqueous HCO_2H to which was added 5 ml of 30% H_2O_2. After 3 hr at room temperature the reaction mixture was concentrated with the repeated addition of H_2O to remove both HCO_2H and H_2O_2. The final residue was taken up in 4 ml of H_2O and 50 ml of EtOH was gradually added to effect precipitation. The resulting white precipitates were collected by centrifugation, washed twice with a small amount of EtOH, and with ether. After drying the precipitates *in vacuo* VII was obtained as a white powder, 0.67 g, 84%. The powder contained a small amount of impurities which was detected on paper chromatogramm (*Rf* 0.60, EtOH–1M NH_4OAc, pH 7, 5: 2, *Rf* of VII, 0.52). UV and NMR measurements (NMR, δ, 8.16) of the crude VII are indicative of contamination with O-formylated derivative of VII. Treatment of the crude VII with 10% NH_4OH for 1 hr at room temperature and crystallization from aqueous EtOH furnished pure VII, mp 161° (shrink, colored at 220°). *Anal.* Calcd. for $C_9H_{11}O_9N_2SK \cdot H_2O$: C, 28.42; H, 3.44; N, 7.36; S, 8.43. Found: C, 28.65; H, 3.48; N, 7.50; S, 8.61. UV $\lambda_{max}^{H_2O}$ nm (ε): 264 (9500), $\lambda_{min}^{H_2O}$ nm (ε): 232.5 (2800), λ_{max}^{1NNaOH} nm (ε): 264 (7400), λ_{min}^{1NNaOH} nm (ε): 230 (6000). CD spectra nm (θ): through 288 (−400), 281.5 (0), peak 258 (+3200), 237 (0), trough 215 (−7700). NMR: see Table I. Mobility on a paper electrophoresis (0.01M ammonium formate buffer, pH 3.5, 600 volts, 1 hr): +6.3 cm; orotidine, +6.3 cm; uridine, 0 cm.

Acknowledgement This work was supported, in part, by the Grant-in-Aid for Scientific Research from the Ministry of Eduction, which is gratefully acknowledged. The NMR, mass and elemental analyses were performed by the members of the Central Analytical Room of this faculty to whom our thanks are due.