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The Reaction of 6-Phenylthiouridine with Sulfur Nucleophiles: A Simple and Regiospecific Preparation of 6-Alkylthiouridines and 6-Alkylthiouridylic Acids

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6-Alkylthiouridines and 6-alkylthiouridylic acids were synthesized from the corresponding 6-phenylthiouridine derivatives *via* a regiospecific nucleophilic reaction.

Keywords——uridine; 6-alkylthiouridine; 6-alkylthiouridylic acid; regiospecific reaction; ^{1}H-NMR

For the preparation of pyrimidine nucleosides, the ribosidation method has been extensively studied.[1] This method is known to be applicable to C-5 substituted pyrimidines but, in general, not to C-6 substituted ones. In the case of the latter, the preferential formation of the N-3 ribosylated product usually takes place, presumably due to the steric hindrance of the substituent in the C-6 position. For example, Winkley and Robins noted that treatment of the bistrimethylsilyl derivative of 6-methylthiouracil with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide in acetonitrile gave 6-methylthio-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)uracil as the sole product in 72% yield.[2] To eliminate such undesired reactions, there is a need for a procedure to transform naturally occurring pyrimidine nucleosides to the C-6 substituted analogues.

We recently reported[3] that the lithiation of 2′,3′-*O*-isopropylidene-5′-*O*-methoxymethyluridine with lithium diisopropylamide occurs at its C-6 position in a regiospecific manner and that the reaction of the resulting dianion with various types of electrophiles represented a new and general route to 6-substituted uridines.

Among the electrophiles employed in this reaction, diphenyl disulfide was quite effective, producing 2′,3′-*O*-isopropylidene-5′-*O*-methoxymethyl-6-phenylthiouridine (**1**) in high yield.[3] On the other hand, the reaction with an alkyl counterpart such as dimethyl disulfide gave a complex mixture of products, from which the 6-methylthio derivative (**2**) was isolated in only 9.3% yield.[4] This prompted us to devise an alternative route to the 6-alkylthiouridine derivatives. In this paper, we describe an efficient method for synthesizing 6-alkylthiouridines and 6-alkylthiouridylic acids which have, so far, been difficult to obtain.

A fairly recent study by one of the authors showed that the 5-bromo uridine derivative **3** is susceptible to nucleophilic reaction with benzyl mercaptan to yield the 6-benzylthio (**6**:

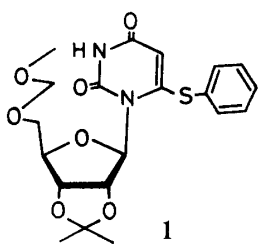

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Fig. 1

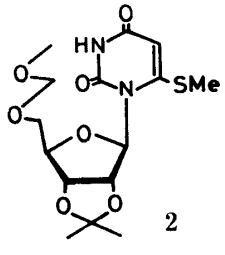

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Fig. 2

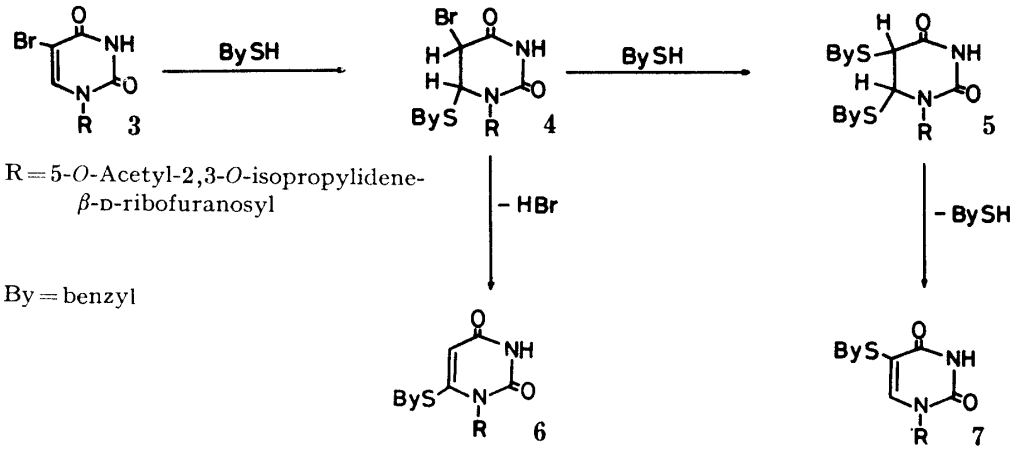


Chart 1

34%) and 5-benzylthio (**7**: 40%) derivatives.[5] The formation of **7** could be attributed to nucleophilic displacement (**4**→**5**) in the intermediate **4** which competes with elimination of hydrogen bromide from **4** (Chart 1). Based on the above mechanistic interpretation, we reasoned that a uridine derivative bearing a suitable leaving group at the C-6 position should furnish the desired 6-sulfur substituted product exclusively because displacement at C-5 by the sulfur nucleophile could be avoided in the 5,6-dihydro intermediate, and it appeared that the 6-phenylthiouridine derivative **1** would fulfil this requirement.

We first examined the reaction of **1** with methylmercaptide anion. When **1** in *N*,*N*-dimethylformamide (DMF) was treated with 5 eq of aqueous sodium methylmercaptide (sodium methanethiolate) at room temperature for 1.5 h, the 6-methylthio derivative (**2**) was obtained in 91.5% yield after extraction with ethyl acetate followed by chromatographic purification on a silica gel column. The ^{1}H-nuclear magnetic resonance (NMR) spectrum of **2** ($CDCl_3$ δ: H-1′ 6.07 doublet; H-5 5.49 singlet; SMe 2.48 singlet) was consistent with the expected structure and indistinguishable from that of a previously prepared sample.[4] As expected, the C-5 substituted product was hardly detectable by careful thin-layer chromatographic (TLC) analysis (benzene: AcOEt=1: 1) and ^{1}H-NMR study (100 MHz, in $CDCl_3$) of

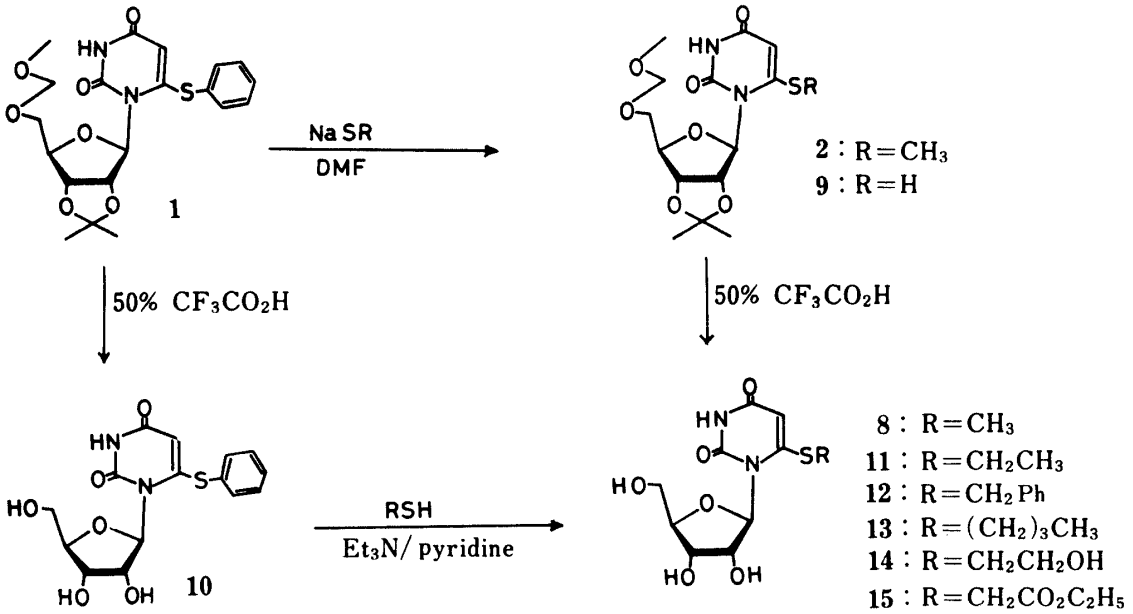
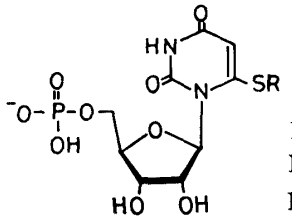


Chart 2

the crude **2**. The structure of **2** was further confirmed by treating it with 50% aqueous trifluoroacetic acid to give the corresponding free nucleoside **8** (80.8%) whose physical data have been reported.[5] Under similar conditions, a crystalline 6-mercaptouridine derivative **9** (mp 190—191°C) was also prepared from **1** in quantitative yield by the use of sodium hydrosulfide. This provides a more convenient method for the 6-mercaptouridine derivative than the previously reported sulfhydrolysis of 6-benzylthiouridine[5] and 6-methylaminouridine[6] derivatives.

The free 6-phenylthiouridine (**10**)[3] also followed this reaction course, providing another route to the free6-alkylthiouridines. The reactions of **10** with alkyl mercaptans were carried out in pyridine in the presence of triethylamine at room temperature. Under these conditions, the 6-ethylthio (**11**), 6-benzylthio (**12**), and 6-butylthio (**13**) derivatives were obtained in 90.5, 77.7, and 93.8%, respectively. We believe that this constitutes an effective route to the free 6-alkylthiouridines, since only mild reaction conditions and simple isolation procedures (evaporation of the solvent and washing of the residue with benzene) are required. Functionalized mercaptans such as β-mercaptoethanol and ethyl thioglycolate also appear to be effective, providing **14** and **15** in good yields. In contrast, the reaction of **10** with *tert*-butyl and isobutyl mercaptans failed. Steric repulsion between the 6-phenylthio group and these rather bulky reagents when adding to the 5,6-double bond is presumably responsible for the above failures.



16 : R = Ph
17 : R = H
18 : R = CH_3

Fig. 3

We next investigated the displacement of the 6-phenylthio group at the nucleotide level, since the reaction proceeded under very mild conditions as mentioned above. Selective phosphorylation of the 5′-hydroxyl group in **10** was achieved according to the published procedure[7] to give 6-phenylthio-5′-UMP (**16**) in 62% yield. When **16** was treated with sodium hydrosulfide in DMF at room temperature, the desired 6-mercapto-5′-UMP (**17**) was obtained in 92% yield. Further derivatization of **17** was carried out by alkylation with methyl iodide to provide 6-methylthio-5′-UMP (**18**).

TABLE I. ^{1}H-NMR Data for 6-Alkylthiouridines in DMSO-d_6 (after addition of D_2O)

C-6 substituent	H-5	H-1′ ($J_{1',2'}$)	H-2′	H-3′	H-4′	CH_2-5′	Other protons
SCH_3 (**8**)	5.45	5.67 (3.4 Hz)	4.57	4.07	3.68	~3.39	CH_3 2.50
SPh (**10**)	4.67	5.79 (3.9 Hz)	4.65	4.12	3.73	~3.40	Ph 7.63
SCH_2CH_3 (**11**)	5.53	5.69 (3.9 Hz)	4.57	4.01	3.71	~3.36	CH_2 3.04, CH_3 1.32
SCH_2Ph (**12**)	5.61	5.69 (3.9 Hz)	4.57	4.06	3.67	~3.40	Ph 7.48—7.31, CH_2 4.33
S $(CH_2)_3CH_3$ (**13**)	5.53	5.72 (3.9 Hz)	4.57	4.08	3.68	~3.40	SCH_2 3.02, $(CH_2)_2$ 1.72—1.31, CH_3 0.92
SCH_2CH_2OH (**14**)	5.58	5.73 (3.4 Hz)	4.57	4.07	——[a]	~3.39	SCH_2 3.12, CH_2OH ——[a]
$SCH_2CO_2C_2H_5$ (**15**)	5.52	5.74 (3.9 Hz)	4.59	——[a]	3.70	~3.36	SCH_2 4.08, $CO_2C_2H_5$ 4.17 and 1.22
CH_3	5.58	5.47 (3.9 Hz)	4.57	4.09	3.69	~3.42	CH_3 2.28
H	5.68	5.78 (4.9 Hz)	4.11	~3.90	——[a]	~3.50	H-6 7.88

a) Not resolved.

The ^{1}H-NMR data for the free 6-alkylthiouridines (**8** and **11**—**15**) involved in the present study are listed in Table I, together with those for 6-phenylthiouridine (**10**), 6-methyluridine, and uridine. It is well known from crystallographic[8] and NMR[9,10] studies that 6-substituted pyrimidine nucleosides prefer the *syn*-conformation: the C-2 carbonyl is situated on the same side as the ribose ring. All the 6-alkylthiouridines, including 6-phenylthiouridine (**10**), listed in Table I display ^{1}H-NMR features similar to those of 6-methyluridine. That is, their H-2′ and H-3′ signals are located downfield compared to those of uridine. This is a well-known charac-

teristic of nucleosides in the *syn*-conformation.[5,10] As can be seen from Table I, change of the alkyl group in the C-6 substituent of these nucleosides has little effect on the chemical shifts of H-5 with the exception of **10**, in which H-5 appears at higher field by *ca.* 0.9 ppm. Examination of a molecular model strongly suggested that the H-5 in **10** can take a position directly above the phenyl ring, and thus, should be shielded by the ring current effect. Our previous finding that the *endo* methyl signal of the isopropylidene group in 2′,3′-*O*-isopropylidene-6-phenylthiouridine is not affected by an anisotropic effect of the C-6 substituent[11] provides further support for the above conformation of **10**.

Experimental

Melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. ^{1}H-NMR spectra were measured with a JEOL JNM-FX 100 NMR spectrometer with an appropriate internal standard: tetramethylsilane (TMS) or sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS). The abbreviations used are as follows: s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; m, multiplet; br, broad. Mass spectra (MS) were taken on a JEOL JMS-D 300 spectrometer. Ultraviolet (UV) spectra were recorded on Hitachi 340 or Shimadzu UV-240 spectrometer. Column chromatography was carried out on Merck Silica Gel 60 or Florisil (Floridin, Inc.). Column chromatographic purification of 5′-phosphates was performed on DEAE-Cellulofine AL (Chisso, Inc.). TLC was performed on silica gel (Precoated silica gel plate F_{254}, Merck) or Avicel SF (Funakoshi Pharmaceutical, Inc.).

2′,3′-*O*-Isopropylidene-5′-*O*-methoxymethyl-6-methylthiouridine (2)——To a suspension of **1** (500 mg, 1.15 mmol) in 15% aqueous NaSMe (2.69 ml, 5.75 mmol), 0.3 ml of DMF was added. The resulting solution was stirred for 1.5 h at room temperature. After being quenched with AcOH (0.33 ml), the reaction mixture was poured into saline. Extraction with AcOEt followed by chromatographic purification on a silica gel (10 g) column (1% EtOH in $CHCl_3$) gave 394 mg (91.5%) of **2** as a syrup. MS *m/z*: 374/(M^+), 359 (M−15), 158 (B+1). NMR ($CDCl_3$) δ: 1.35 (3H, s, isop.Me), 1.56 (3H, s, isop.Me), 2.48 (3H, s, SMe), 3.36 (3H, s, $CH_2OC\underline{H}_3$), 3.72—3.79 (2H, m, CH_2-5′), 4.20—4.37 (1H, m, H-4′), 4.65 (2H, s, $C\underline{H}_2OCH_3$), 4.88 (1H, dd, H-3′), 5.22 (1H, dd, H-2′), 5.49 (1H, s, H-5), 6.07 (1H, d, J=1.5 Hz, H-1′), 8.02 (1H, br, NH).

6-Methylthiouridine (8)——Treatment of **2** (276 mg) with 50% aqueous CF_3COOH (5 ml) for 2 d at room temperature followed by chromatographic purification (10% EtOH in $CHCl_3$) gave **8** (173 mg, 80.8%), which was crystallized from MeOH: mp 171.5—172.5°C (resolidified at 173.5°C, dec. at 223°C); lit.[5] mp 163—165.5°C (resolidified at 166°C, dec. at 230°C). NMR: see Table I.

2′,3′-*O*-Isopropylidene-5′-*O*-methoxymethyl-6-mercaptouridine (9)——A DMF (5 ml) solution of **2** (500 mg, 1.15 mmol) and 70% NaSH (200 mg, 2.50 mmol) was stirred for 2.5 h at room temperature. After being neutralized with Amberlite IR-120-B (H^+ form), the mixture was evaporated to dryness. Chromatographic purification on Florisil (50% MeOH in CH_2Cl_2) gave 413 mg (100%) of **9**. Crystallization from isopropanol gave an analytical sample: mp 190—191°C. *Anal.* Calcd for $C_{14}H_{20}N_2O_7S$: C, 46.66; H, 5.59; N, 7.77. Found: C, 46.42; H, 5.60; N, 7.53. MS *m/z*: 361 (M+1), 345 (M−15), 144 (B+1). UV $\lambda_{max}^{methanol}$ nm (ε): 316 (23300) and 238 (8400), $\lambda_{min}^{methanol}$ nm (ε): 263 (600) and 222 (5500). NMR (DMSO-d_6) δ: 1.26 (3H, s, isop. Me), 1.46 (3H, s, isop. Me), 3.24 (3H, s, $CH_2OC\underline{H}_3$), 3.40—3.74 (2H, m, CH_2-5′), 3.85—4.04 (1H, m, H-4′), 4.53 (2H, s, $C\underline{H}_2OCH_3$), 4.69 (1H, dd, H-3′), 4.97 (1H, d, H-2′), 5.30 (1H, d, H-5), 7.66 (1H, s, H-1′), 10.13 (1H, br, NH).

6-Phenylthiouridine (10)——An aqueous 50% CF_3CO_2H solution (30 ml) containing **1** (3.0 g, 6.87 mmol) was stirred for 2 d at room temperature. The mixture was then evaporated to dryness and the resulting residue was chromatographed on a silica gel column (5% EtOH in $CHCl_3$) to afford **10** (2.07 g, 85.5%). Crystallization from EtOAc gave an analytical sample: mp 156—159°C. *Anal.* Calcd for $C_{15}H_{16}N_2O_6S$: C, 51.13; H, 4.58; N, 7.95. Found: C, 51.08; H, 4.64; N, 7.77. UV $\lambda_{max}^{methanol}$ nm (ε): 282 (11000), $\lambda_{min}^{methanol}$ nm (ε): 245 (5400). NMR: see Table I.

6-Ethylthiouridine (11)——Et_3N (1.6 ml) and EtSH (0.85 ml) were added to a pyridine (3 ml) solution of **10** (403 mg, 1.14 mmol). After being stirred overnight at room temperature, the reaction mixture was evaporated to dryness. The resulting residue was washed with benzene to leave 314 mg (90.5%) of **11**. Crystallization from H_2O gave an analytical sample: mp 190.5—191.5°C. *Anal.* Calcd for $C_{11}H_{16}N_2O_6S$: C, 43.41; H, 5.30; N, 9.21. Found: C, 43.20; H, 5.27; N, 9.31. MS *m/z*: 286 (M−H_2O), 172 (B+1). UV $\lambda_{max}^{H_2O}$ nm (ε): 283 (14700), $\lambda_{min}^{H_2O}$ nm (ε): 249 (3300). NMR: see Table I.

6-Benzylthiouridine (12)——Et_3N (1.24 ml) and benzyl mercaptan (1.0 ml) were added to a pyridine (3 ml) solution of **10** (302 mg, 0.86 mmol). After being stirred overnight at room temperature, the reaction mixture was evaporated to dryness. The resulting residue was washed with benzene to leave 253 mg (77.7%) of **12**. Crystallization from EtOH gave pure **12**: mp 174.5—176°C; lit.[5] mp 169—170°C, resolidified at 172°C, dec. at 225°C. NMR: see Table I.

6-Butylthiouridine (13)——Et_3N (1.5 ml) and butyl mercaptan (1.2 ml) were added to a pyridine (3 ml)

solution of **10** (385 mg, 1.09 mmol). After being stirred for 2 d at room temperature, the reaction mixture was evaporated to dryness. The resulting residue was washed with benzene to leave 340 mg (93.8%) of **13**. Crystallization from EtOH gave an analytical sample: mp 148.5—150°C. *Anal.* Calcd for $C_{13}H_{20}N_2O_6S$: C, 46.97; H, 6.07; N, 8.43. Found: C, 46.72; H, 6.05; N, 8.59. UV $\lambda_{max}^{methanol}$ nm (ε): 279 (13000), $\lambda_{min}^{methanol}$ nm (ε): 247.7 (3400). NMR: see Table I.

6-(β-Hydroxy)ethylthiouridine (14)——Et_3N (1.4 ml) and 2-mercaptoethanol (0.7 ml) were added to a pyridine (3 ml) solution of **10** (366 mg, 1.00 mmol). After being stirred for 2 h at room temperature, the reaction mixture was evaporated to dryness. The resulting residue was chromatographed on a silica gel column (15% EtOH in $CHCl_3$) to afford 243 mg (75.9%) of **14**. Crystallization from EtOH gave an analytical sample: mp 166—167.5°C. *Anal.* Calcd for $C_{11}H_{16}N_2O_7S$: C, 41.24; H, 5.04; N, 8.75. Found: C, 41.48; H, 5.16; N, 8.63. MS *m/z*: 302 (M−H_2O), 217 (B+CHOH), 188 (B+1). UV $\lambda_{max}^{methanol}$ nm (ε): 279.5 (13300), $\lambda_{min}^{methanol}$ nm (ε): 237.5 (3800). NMR: see Table I.

6-Ethoxycarbonylmethylthiouridine (15)——Et_3N (1.52 ml) and ethyl thioglycolate (1.2 ml) were added to a pyridine (3 ml) solution of **10** (384 mg, 1.09 mmol). After being stirred for 3 h at room temperature, the reaction mixture was evaporated to dryness. The resulting residue was chromatographed on a silica gel column (5% EtOH in $CHCl_3$). This afforded 301 mg (76.3%) of **15**. Crystallization from EtOH gave hygroscopic crystals: mp 112—114°C. UV $\lambda_{max}^{methanol}$ nm: 277.5, $\lambda_{min}^{methanol}$ nm: 245. MS *m/z*: 230 (B+1). NMR: see Table I.

6-Phenylthiouridine 5′-Phosphate (16)——An ice-cooled $(MeO)_3PO$ (2.5 ml) solution of **10** (324 mg, 0.92 mmol) was treated with 0.17 ml (2 equivalents) of $POCl_3$. After being stirred for 6 h at 0°C, the reaction mixture was quenched with saturated $NaHCO_3$ (10 ml). The aqueous solution was washed with two 10 ml portions of ether, acidified with 1 N HCl to pH 5, diluted with H_2O to a volume of *ca.* 400 ml, and applied to a column of DEAE-Cellulofine (bicarbonate form, 2.4×26 cm). Elution of **16** was performed with a linear gradient of 0 to 0.4 M triethylammonium bicarbonate (800 ml each). Fractions of 18 ml were collected. Fractions No. 32—41 were combined and concentrated to give the triethylammonium salt of **16** (6330 ODU at 283 nm, 62%, calculated from ε of **16**=11000). UV $\lambda_{max}^{H_2O}$ nm: 283, $\lambda_{min}^{H_2O}$ nm: 246. NMR (D_2O) δ: 3.90—4.10 (3H, m, CH_2-5′ and H-4′), 4.46 (1H, t, H-3′), 4.83 (1H, dd, H-2′), 5.23 (1H, s, H-5), 6.08 (1H, d, J=2.9 Hz, H-1′), 7.57—7.67 (5H, m, phenyl). *Rf* values: see Table II.

6-Mercaptouridine 5′-Phosphate (17)——NaSH (100 mg) was added to a DMF (10 ml) solution of **16** (triethylammonium salt, 2100 ODU at 283 nm). After being stirred overnight, the reaction mixture was diluted with H_2O (300 ml), acidified with 1 N HCl to pH 4, and bubbled through with N_2 gas to remove H_2S. The resulting cloudy solution was applied to a column of DEAE-Cellulofine (bicarbonate form, 2.4×9 cm). The column was washed with H_2O (500 ml). Elution of **17** was performed with a linear gradient of 0 to 0.4 M triethylammonium bicarbonate, and 15 ml fractions were collected. Fractions No. 28—49 were combined and concentrated to give the triethylammonium salt of **17** (4725 ODU at 317 nm, 92%, calculated from ε of **17**=23300). UV $\lambda_{max}^{H_2O}$ nm: 317 and 233, $\lambda_{min}^{H_2O}$ nm: 262 and 220. *Rf* values: see Table II.

6-Methylthiouridine 5′-Phosphate (18)——A 1.2 ml aliquot of 1 N NaOH and 1.0 ml of MeI were added with stirring to a MeOH (20 ml) solution of **17** (triethylammonium salt, 5100 ODU at 317 nm). After 1 h at room temperature, the reaction mixture was diluted with H_2O (300 ml), acidified with 1 N HCl to pH 6, and applied to a column of DEAE-Cellulofine (bicarbonate form, 2.4×7 cm). Elution of **18** was performed with a linear gradient of 0 to 0.3 M triethylammonium bicarbonate (400 ml each). Fractions of 15 ml were collected. Fractions No. 21—39 were combined and concentrated to give the triethylammonium salt of **18** (3170 ODU at 282 nm, 94%, calculated from ε of **18**=15400). UV $\lambda_{max}^{H_2O}$ nm: 282, $\lambda_{min}^{H_2O}$ nm: 248. NMR (D_2O) δ: 2.56 (3H, s, SMe), 3.92—4.16 (3H, m, CH_2-5′ and H-4′), 4.45 (1H, t, H-3′), 4.84 (1H, dd, H-2′), 5.65 (1H, s, H-5), 5.98 (1H, d, J=2.9 Hz, H-1′). *Rf* values: see Table II.

TABLE II. *Rf* Values of Compounds **16—18**

Compound	Solvent system[a]		
	A	B	C
5′-UMP	0.40	0.15	0.06
16	0.63	0.42	0.12
17	0.30	0.08	0.06
18	0.46	0.20	0.08

a) A, EtOH-1M NH_4OAc (5:4); B, EtOH-1M NH_4OAc (5:2); C, isopropanol-NH_4OH-H_2O (7:1:2).

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