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Syntheses of Potential Antimetabolites. XX.*,[1] Syntheses of 5-Carbomethoxymethyl- and 5-Methylaminomethyl-2-thiouridine (The "First Letters" of Some Anticodons) and Closely Related Nucleosides from Uridine

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A procedure for the preparation of 5-substituted 2-thiouridines from uridine has been developed, whereby 5-carbomethoxymethyl- (**8b**) and 5-methylaminomethyl-2-thiouridine (**10**) (each may be the first letter of certain anticodons of some transfer ribonucleic acids) and the closely related nucleosides [*e.g.*, 5-hydroxymethyl-2-thiouridine (**11**), 5-cyanomethyl-2-thiouridine (**6b**), and 5-carbomethoxymethyluridine (**8b**)] were prepared in satisfactory yields. Acylation of 5-methylaminomethyl-2-thiouridine (**10**) was also examined and 5-(N-methylbenzamido)methyl-2-thiouridines (**12**) was prepared and characterized.

Some 5-substituted 2-thiouridines such as 5-carbomethoxy- (**8b**) and 5-methylaminomethyl-2-thiouridine (**10**) have been isolated from transfer ribonucleic acids (t-RNA)[3] and were found to be located at the first position of the anticodon of the transfer RNA.[4,5] These 2-thiouridines have become of interest because of the unique position of their occurrence in the t-RNA and their possible role in translating genetic information in the protein biosynthesis.[6] Development of efficient synthetic procedures for these 2-thiouridines (**8b** and **10**) by the unambiguous chemical means is of urgent need in order to study their physical and biological properties as well as their fine structures.

The nucleoside (**8b**) has been synthesized by the condensation of a blocked glycosyl halide with an appropriate base,[7] whereas the chemical synthesis of **10** has never been reported.

The present paper reports the chemical synthesis of **8b** and **10** and the closely related nucleosides from 2′,3′-O-isopropylidene-2-thiouridine (**1b**)[8] which may be easily obtainable from uridine by four steps.

The outline of the synthetic scheme which we adopted is given in Chart 1 and 2 where one of the key steps consists in the introduction of hydroxymethyl group into the 5-position of uridine or 2-thiouridines which has been originally developed by Scheit.[9] He has prepared 2′,3′-O-isopropylidene-5-hydroxymethyluridine (**2a**) by treatment of 2′,3′-O-isopropyliden-

* Dedicated to the memory of Prof. Eiji Ochiai.

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3) L. Baczynski, K. Biemann, and R.H. Hall, *Science*, **159**, 1481 (1968); J.A. Carbon, H. David, and M.H. Studier, *Science*, **161**, 1146 (1968).
4) M. Yoshida, K. Takeishi, and T. Ukita, *Biochem. Biophys. Res. Commun.*, **39**, 852 (1970).
5) Z. Ohashi, M. Saneyoshi, F. Harada, H. Hara, and S. Nishimura, *Biochem. Biophys. Res. Commun.*, **40**, 866 (1970).
6) F.H. Crick, *J. Mol. Biol.*, **19**, 548 (1966).
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8) T. Ueda and S. Shibuya, *Chem. Pharm. Bull.* (Tokyo), **18**, 1076 (1970).
9) K.H. Scheit, *Chem. Ber.*, **99**, 3884 (1966).

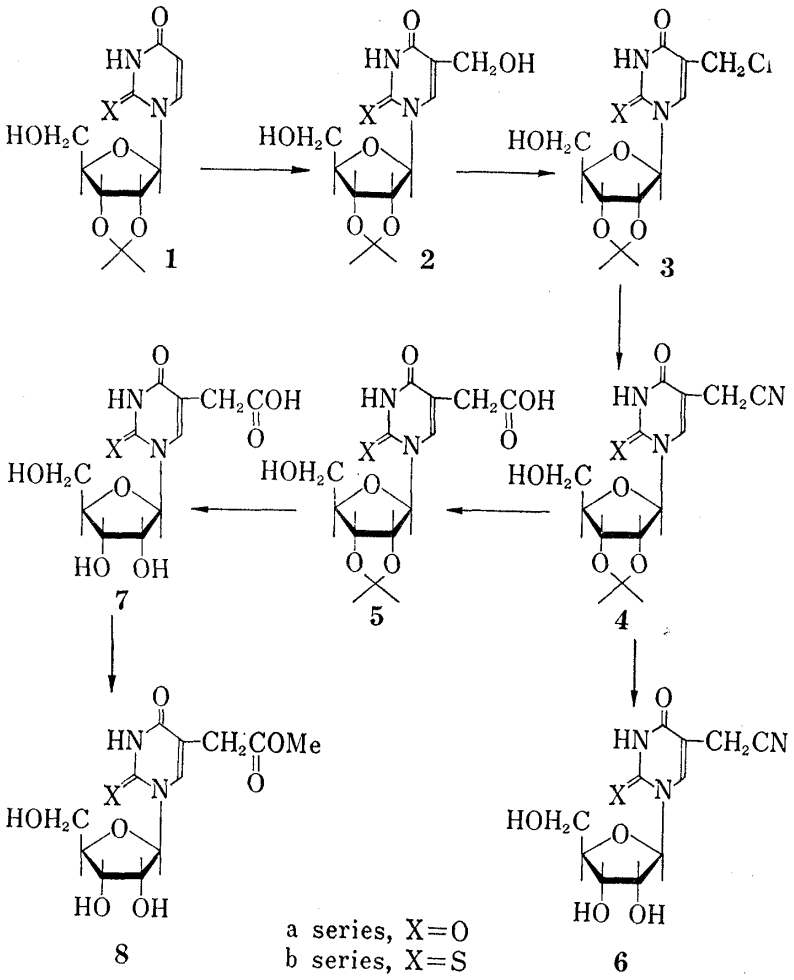

Chart 1

euridine (**1a**) with paraformaldehyde in alkaline media.[9] Another key step consists in the selective chlorination of 5-hydroxymethyl group, other hydroxyl groups remaining intact. To this end we adopted the method which Brossmer and Röhm have developed for the preparation of 3′,5′-di-O-toluyl-5-chloromethyl-2′-deoxyuridine from 3′,5′-di-O-toluyl-5-hydroxymethyl-2′-deoxyuridine.[10]

We first deal with the preparation of 5-cyanomethyluridine (**6a**) and 5-carbomethoxyuridine (**8a**). Nucleoside (**2a**)[9] was treated with hydrogen chloride in dioxane at 0—5° for 2 hr to give 2′,3′-O-isopropylidene-5-chloromethyluridine (**3a**), contaminated with a considerable amount of a de-acetonized product. After being subjected to acetonization, the product (**3a**) was treated without purification with potassium cyanide in aqueous solution at room temperature for 2 hr to afford 2′,3′-O-isopropylidene-5-cyanomethyluridine (**4a**) in 89% yield. Treatment of the latter (**4a**) with 20% acetic acid at about 100° for 2 hr afforded 5-cyanomethyluridine (**6a**) in 91% yield. Hydrolysis of **4a** with 1N KOH at 80° for 8 hr and subsequently with a Dowex resin (H^+ form) gave rise to 5′-carboxymethyluridine (**7a**) in 75% overall yield. Methylation of **7a** was achieved by treatment with anhydrous methanol in the presence of a catalytic amount of conc. H_2SO_4. Melting point as well as nuclear magnetic resonance (NMR) spectral data of the above samples (**7a** and **8a**) were found to be in good agreement with reported data.[11]

10) R. Brossmer and E. Röhm, *Z. Physiol. Chem.*, **348**, 1431 (1967).
11) J.D. Fissekis and F. Sweet, *Biochemistry*, **9**, 3136 (1970).

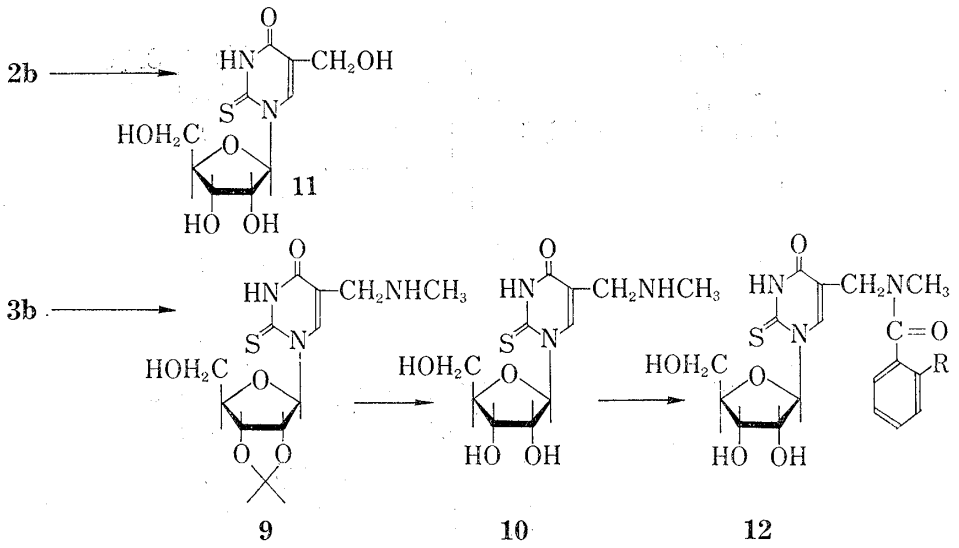

Chart 2

We secondly deal with the preparation of 5-hydroxymethyl-2-thiouridine (**11**) and 5-methylaminomethyl-2-thiouridine (**10**). Nucleoside (**1b**) was treated with paraformaldehyde in 0.5N sodium hydroxide solution to afford a mixture of **2b** and **2a**. In order to prevent undesirable hydrolysis of the 2-thio-group, some attempts have been made and eventually it was found that the organic base such as triethylamine acts as a suitable base for the hydroxymethylation. Thus, nucleoside (**1b**) was heated with paraformaldehyde in 0.5M triethylamine at 60° for 9 hr to give **2b** in 80% yield after crystallization from ethanol. The structural confirmation rests upon the NMR spectral data (in D_2O) which showed the presence of two-proton singlet at δ 4.38 (attributable to methylene attached to the uracil nucleus) and the absence of a signal due to H-5 which may usually appear at around 7 ppm. The ultraviolet (UV) spectra are quite similar to those of 2-thiouridine. Elemental analysis was in good agreement with its theoretical values.

Treatment of **2b** with 20% acetic acid gave rise to 5-hydroxymethyl-2-thiouridine. The structural confirmation rests upon combustion values as well as UV spectra. Treatment of **2b** with hydrogen chloride and subsequent re-acetonization with acetone afforded 2′,3′-O-isopropylidene-5-chloromethyl-2-thiouridine (**3b**) which in turn was treated with sodium cyanide. The corresponding cyanomethyl derivative (**4b**) could be obtained in excellent yield as crystalline product. The infrared (IR) spectra of **4a** showed a band at 2260 cm^{-1} due to –CN streching vibration. The NMR spectrum in D_2O showed a signal at 4.01 ppm which appears upfield by 0.27 ppm as compared with the corresponding methylene signal in **2b**. Treatment of **4b** with aqueous acetic acid gave rise to 5-cyanomethyl-2-thiouridine (**6b**) in 80% yield. Compound **4b** and **6b** had the UV spectra similar to those of 2-thiouridine. The elemental analyses of both compounds were also in good agreement with respective theoretical values.

We next move on to the synthesis of 5-carbomethoxymethyl-2-thiouridine (**8b**). The nucleoside **4b** was treated under comparatively mild conditions[12] (0.5N NaOH at 70° for 6 hr) and the reaction was monitored by paper electrophoresis which showed the presence of a predominant amount of the product having large mobility (presumably **5b**), a small amount of the starting material still remaining intact. Prolonged heating at the same temperature

12) The reaction of **4b** with 1N NaOH (80° for 6 hr) was followed by paper electrophoresis which showed (after 6 hr) the presence of two spots arising from products having negative charge at pH 8. One of these was tentatively assigned as **7a** because this had the same mobility and the same UV spectra as **7a**. Another spot had larger mobility and had the UV absorption spectra, typical of 2-thiouridines.

resulted in partial hydrolysis of the product to afford a mixture of **5a** and **5b**. Preparation of **5b**, free of **4b** could be finally achieved by the aid of a phospho-cellulose column. Compound **5b** could be isolated from the faster travelling fraction. However, this sample was now contaminated with a small amount (*ca.* 10%) of another by-product, 5-carboxymethyl-2-thiouridine (**7b**). In view of such a complex separation problem, attempts to obtain a pure sample of **5b** were abandoned and instead the mixture as such was treated with 20% acetic acid to afford **7b** in pure state (after crystallization from methanol). Methylation of the latter with anhydrous methanol in the presence of a catalytic amount of conc. H_2SO_4 afforded 5-carbomethoxymethyl-2-thiouridine (**8b**) in 70% yield. The structural confirmation rests upon spectral (UV and NMR) data as well as its combustion values. Thus the NMR spectrum in DMSO-d_6 showed signals at 3.32 ppm (methoxycarbonyl), 4.02 ppm (methylene at position 5), 6.55 ppm (anomeric proton), and 8.12 ppm (H-6). Its melting point was also as reported.[7]

A dioxane solution of **3b** was treated with methylamine in anhydrous methanol at room temperature overnight. The reaction was monitored by thin-layer chromatography (TLC) over silica gel (solvent system: $CHCl_3$–MeOH 7: 1); the reaction mixture was found to contain two products, **9** (a major product) and **10** (deblocked nucleoside, a minor product). Each product was separated by the aid of a column chromatography over silica gel. Compound **9** was crystallized from methanol. The NMR spectrum in D_2O was found to be compatible with the structure assigned. Deblocking with 10% aqueous acetic acid at boiling water temperature gave rise to **10** as crystals. This synthetic sample (**10**) was found identical with a sample of natural origin isolated from *Escherichia coli*[13] on the criteria of the UV spectral and the chromatographic behavior (five different solvent systems were used).

The present work is pertinent to that of Cedergren and his coworkers who have benzoylated **10** (prepared from t-RNA) at pH 8.0 to obtain monobenzoylated product whose structure has been tentatively assigned as 5-(N-methylbenzamido)-methyl-2-thiouridine (**12**, R–H) without unequivocal proof.[14]

In order to obtain an information on the behavior of **10** toward acylation and to confirm the above structural assignment, the synthetic sample of **10** was treated with 2 equivalents of phthalic anhydride in aqueous solution at pH 8 at room temperature. The paper electrophoretic examination of the reaction showed that the mixture contained at least two products, each having similar mobility, which could scarecely be separated by a chromatography over diethylaminoethyl (DEAE)-cellulose. Thus O-acylated nucleoside (s) could be isolated from the faster travelling fraction which afforded on heating with 0.5N sodium hydroxide solution at 60° for 6 minutes, **10** and phthalic acid.[15] Another product could be obtained from the slower travelling fraction and has been tentatively assigned the N-acylated nucleoside structure (**12**, R=COOH).[15] Attempted crystallization of the latter failed because of its unstable nature.

We then tried benzoic anhydride as acylating agent. In addition, in view of the information[15] that the selective hydrolysis of O-acyl linkage in acylated products may be effected, in this case the selective alkaline hydrolysis was performed prior to the separation of the products in the hope that the separation of N-benzamidoderivative from O-benzoate might be facilitated. This device was found to be rewarding. Thus, compound **10** was mixed with 5 equivalents of benzoic anhydride in aqueous dimethyl formamide (DMF) at pH 8 and subsequently the reaction mixture was allowed to stand at pH 12 for 2 hr at room temperature to effect hydrolysis of O-benzoate linkage alone. After work-up, compound **12** (R=H) was

13) H. Kasai, personal communication.
14) R.J. Cedergren, N. Beauchemin, and J. Toupin, *Biochemistry*, **12**, 4566 (1973).
15) Under the same conditions, the product which was obtained from the slower traveling fraction remained unchanged. These data coupled with the UV spectral data (both had the same UV spectra) strongly suggested that the former fraction contained O-acylated nucleoside (s) and the latter contained N-monoacylated nucleoside.

purified by a column chromatography over silica gel. The structure was confirmed by both elemental analysis and spectrometry. Thus NMR spectrum of **12** (R=H) in DMSO-d_6 showed signals at 2.90 ppm (N-CH_3), 4.01 ppm (methylene attached to the thiouracil ring), 6.60 ppm (anomeric), 7.50 ppm (aromatic), and 8.14 ppm (H-6). IR spectrum showed the presence of bands at 1660, 1600 and 1575 cm^{-1}, and the absence of a band due to ester group. UV spectra of **12** (R=H) was found to be similar to those of **10** at both pH 7 and 12, indicating that no remarkable change in the chromophore took place in the benzoylation and the subsequent hydrolysis. In contrast, at pH 2 the discernible difference could be observed between the UV spectra of **10** and **12** (see Fig. 1 and 2).

These data coupled with its combustion values strongly support that compound **12** and accordingly the sample of Cedergren and his coworkers (*vide supra*) are indeed 5-(N-methylbenzamido)methyl-2-thiouridine.

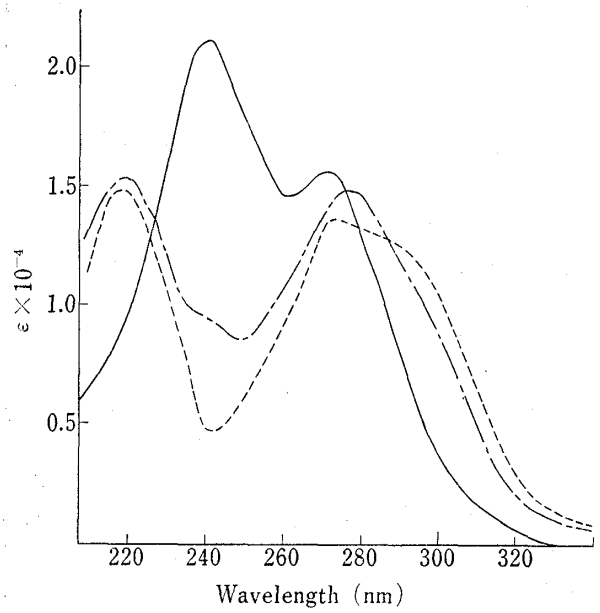

Fig. 1. UV Spectra of 5-Methylaminomethyl-2-thiouridine (**10**)

--------: pH 2.0 ——: pH 7.0 ——: pH 12.0

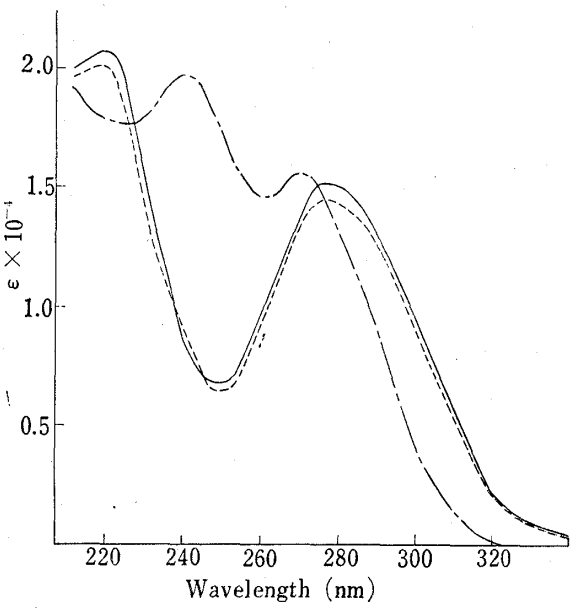

Fig. 2. UV Spectra of 5-(N-Methylbenzamido)-methyl-2-thiouridine (**12**)

--------: pH 2.0 ——: pH 7.0 ——: pH 12.0

Experimental

NMR spectra were determined on a Hitachi R-24 spectrometer. UV and IR measurements were performed on a Hitachi Spectrometer Model 3T and a JASCO Infrared Spectrophotometer DS-701, respectively. Paper electrophoresis was carried out on Toyo Roshi No. 51A impregnated with 0.05M triethylammonium bicarbonate (pH 8.0) using 700 volts. Paper chromatography was carried out by the ascending technique on Toyo Roshi No. 51A paper using the folloing system: isopropanol–28% NH_4OH–H_2O (7:1:2).

2′,3′-O-Isopropylidene-5-hydroxymethyl-2-thiouridine (2b)——A mixture of **1b** (2.00 g) and paraformaldehyde (0.6 g) in 0.5M triethylamine (20 ml) was heated at 60° for 9 hr in a sealed flask. The solution was concentrated to dryness and the residue was crystallized from ethanol to yield, **2b**, mp 176—176.5°. Yield was 1.78 g (81%). *Anal.* Calcd. for $C_{13}H_{18}O_6N_2S$: C, 47.27; H, 5.49; N, 8.49; S, 9.69. Found: C, 47.18; H, 5.43; N, 8.37; S, 9.87. UV $\lambda_{max}^{pH\ 2}$ nm (ε): 220 (15900), 278.5 (15900). $\lambda_{max}^{pH\ 7.5}$ nm (ε): 221 (15900), 277 (16800). $\lambda_{max}^{pH\ 12}$ nm (ε): 241 (21600), 272 (15400). NMR (D_2O) δ: 1.40, 1.63 (6H, s, isopropylidene), 4.38 (2H, methylene attached to heterocycle), 7.08 (1H, d, J=2 Hz, 1′-H), 7.85 (1H, s, H-6).

2′,3′-O-Isopropylidene-5-cyanomethyluridine (4a)——A suspension of **2a** (13.0 g) in dry dioxane saturated with HCl at 0° was magnetically stirred for 2 hr at 0—5°. The resulting clear solution was concentrated to a volume of about 30 ml *in vacuo* below 25°. There was then added acetone (50 ml) and the mixture was allowed to stand at room temperature for 1 hr. The solution was then concentrated to dryness and the remaining HCl was almost completely removed by repeating addition and subsequent evaporation of dioxane. The final residue which was dissolved in dioxane (100 ml) was added to an aqueous solution (130 ml) of KCN

(5.0 g) below 10°. The mixture was allowed to stand at room temperature for 2 hr and neutralized with a resin (Dowex 50 × 8, H^+ form). After filtration, the filtrate was evaporated to dryness *in vacuo*. The residue was crystallized from water to yield **4a**, mp 194°. Yield was 12.6 g (89%). *Anal.* Calcd. for $C_{14}H_{17}O_6N_3 1/2 H_2O$: C, 50.60; H, 5.46; N, 12.65. Found: C, 50.63; H, 5.56; N, 12.73. IR ν_{max}^{Nujol} cm^{-1}: 2360.

5-Cyanomethyluridine (6a)——A solution of **4a** (683 mg) in 20% AcOH (10 ml) was heated in a boiling water bath for 2 hr. The solution was evaporated to dryness *in vacuo*. The remaining acetic acid was removed by repeating addition and evaporation of EtOH. The residue was crystallized from H_2O–EtOH to afford **6a**, mp 227°. Yield was 521 mg (92%). *Anal.* Calcd. for $C_{11}H_{13}O_6N_3$: C, 46.64; H, 4.63; N, 14.84. Found: C, 46.38; H, 4.62; N, 14.98.

5-Carboxylmethyluridine (7a)——A solution of **4a** $1/2H_2O$ (3.41 g) in 1N KOH (40 ml) was heated at 80° for 8 hr. The cooled solution was put on the top of a column (Dowex 50 W, H^+ form) and the column was washed with H_2O. During the passage, deacetonization was accompanied. The eluate containing the desired product was collected and concentrated to a volume of about 10 ml. The solution was heated in a boiling water bath for 4 hr. The solution was then evaporated to dryness *in vacuo*. The residue was crystallized from EtOH–H_2O to afford **7a**, mp 241°. Yield was 2.25 g (75%). *Anal.* Calcd. for $C_{11}H_{14}O_8N_2$: C, 43.71; H, 4.67; N, 9.27. Found: C, 43.63; H, 4.76; N, 9.35. UV λ_{max} nm: 265.5 (pH 1.0), 267 (pH 7.0), 267 (pH 11). NMR (DMSO-d_6) δ: 3.85 (2H, s, $-CH_2CO-$), 5.92 (1H, d, J=4.5 Hz, 1′-H), 8.10 (1H, s, H-6).

5-Carbomethoxymethyluridine (8a)——A solution of **7a** (750 mg) in MeOH (9 ml) containing 98% H_2SO_4 (0.5 ml) was refluxed for 20 hr. The cooled solution was neutralized with powdered $BaCO_3$ (3.8 g) and filtered. The filtrate was evaporated to dryness. The residue was crystallized from MeOH–EtOEt to afford **8a**, mp 163°. Yield was 690 mg (88%). *Anal.* Calcd. for $C_{12}H_{16}O_8N_2$: C, 45.57; H, 5.10; N, 8.86. Found: C, 45.62; H, 4.86; N, 8.81. NMR (DMSO-d_6) δ: 3.41 (3H, s, OCH_3), 4.02 (2H, s, $-CH_2CO-$), 5.94 (1H, d, J=4.0 Hz, 1′-H), 8.18 (1H, s, H-6).

2′,3′-O-Isopropylidene-5-chloromethyl-2-thiouridine (3b)——A mixture of **2b** (2.5 g) in dry dioxane (60 ml), saturated with HCl was stirred for 1.5 hr in an ice-bath. The resulting solution was concentrated to dryness *in vacuo* below 25°. There was then added acetone (50 ml) and the mixture was allowed to stand at room temperature for 1 hr. The solution was concentrated to dryness *in vacuo*. The remaining HCl was removed by repeated addition and evaporation of dioxane. The residue was dissolved in dioxane (30 ml) and the solution was used for the preparation of **4b** and **9**.

2′,3′-O-Isopropylidene-5-cyanomethyl-2-thiouridine (4b)——A dioxane solution of **3b** (prepared from 2.5 g of **2b** as above) was mixed with 50% EtOH (50 ml) containing NaCN (1.0 g) and allowed to stand for 1.5 hr at room temperature. The solution was neutralized with AcOH and evaporated to dryness *in vacuo*. The residue was extracted with hot $CHCl_3$. The solvent was then evaporated to dryness and the resulting residue was crystallized from MeOH to afford **4b**, mp 168—169°. Yield was 2.1 g (82%). *Anal.* Calcd. for $C_{14}H_{17}O_5N_3S$: C, 49.55; H, 5.05; N, 12.39; S, 9.45. Found: C, 49.62; H, 4.92; N, 12.12; S, 9.36. IR ν_{max}^{Nujol} cm^{-1}: 2260 (–CN).

5-Cyanomethyl-2-thiouridine (6b)——A solution of **4b** (100 mg) in 20% AcOH (5 ml) was heated in a boiling water bath for 2 hr and concentrated to dryness. The residue was crystallized from MeOH to afford **6b**, mp 198—200°. Yield was 71 mg (80%). *Anal.* Calcd. for $C_{11}H_{13}O_5N_3S$: C, 44.15; H, 4.38; N, 14.04; S, 10.71. Found: C, 43.92; H, 4.56; N, 14.13; S, 10.67. UV $\lambda_{max}^{pH\ 7.0}$ nm: 221 and 277; $\lambda_{max}^{pH\ 12}$ nm: 241 and 271.

5-Carboxymethyl-2-thiouridine (7b)——A solution of **4b** (1.65 g) in 0.5N NaOH (10 ml) was heated at 70° for 6 hr. The cooled solution was put on the top of a column (phospho-cellulose, H^+ form, column size, 3.0 × 30 cm) and the column was washed with water until the eluate became neutral. The acidic eluate was combined and evaporated to dryness. The residue was dissolved in 20% AcOH (10 ml) and the solution was heated at 100° for 3 hr. The cooled solution was evaporated to dryness. The remaining AcOH was almost completely removed by repeated addition and evaporation of EtOH. The final residue was crystallized from MeOH to afford **7b**, mp 206—207°. Yield was 1.13 g (73%). *Anal.* Calcd. for $C_{11}H_{14}O_7N_2S$: C, 41.50; H, 4.43; N, 8.80; S, 10.07. Found: C, 41.29; H, 4.41; N, 8.62; S, 10.34. NMR (DMSO-d_6) δ: 4.05 (2H, s, $-CH_2CO-$), 6.62 (1H, d, J=2 Hz, 1′-H), 8.15 (1H, s, H-6).

5-Carbomethoxymethyl-2-thiouridine (8b)——A solution of **7b** (950 mg) in dry MeOH (20 ml) containing conc. H_2SO_4 (0.6 ml) was refluxed for 10 hr. The cooled solution was neutralized with powdered $BaCO_3$ (4.0 g) and filtered. The filtrate was concentrated to dryness. The residue was crystallized from EtOH–EtOEt to afford **8b**, mp 195—197°. Yield was 690 mg (70%). *Anal.* Calcd. for $C_{12}H_{16}O_7N_2S$: C, 43.37; H, 4.85; N, 8.43; S, 9.65. Found: C, 43.18; H, 4.92; N, 8.22; S, 9.81. UV λ_{max} nm: 221, 277 (pH 7.0) and 242, 271 (pH 12.0). NMR (DMSO-d_6) δ: 3.32 (3H, s, CH_3O-), 4.02 (2H, s, $-CH_2CO-$), 6.55 (1H, d, J=2 Hz, 1′-H), 8.12 (1H, s, H-6).

2′,3′-O-Isopropylidene-5-methylaminomethyl-2-thiouridine (9)——A dioxane solution of **3b**, prepared from **2b** (4.38 g), was mixed with a solution of MeOH–CH_3NH_2 (1:1) below −10° in a stoppered flask was allowed to stand at room temperature overnight. The residue obtained after concentration of the solutions was dissolved in MeOH and applied to a column (silica gel, 150 g) and the column was washed with $CHCl_3$–MeOH (5:1). Eluate containing **9** was collected and evaporated to dryness. The residue was crystallized from MeOH to afford **9**, mp 163—164°. Yield was 2.84 g (62%). *Anal.* Calcd. for $C_{14}H_{21}O_5N_3S$: C, 48.96;

H, 6.16; N, 12.24; S, 9.34. Found: C, 49.02; H, 6.29; N, 12.28; S, 9.49. NMR (D_2O) δ: 1.44, 1.66 (6H, s, isopropylidene), 2.78 (3H, s, N–CH_3), 4.07 (2H, s, –CH_2N–), 6.69 (1H, d, J=2 Hz, 1′-H), 8.28 (1H, s, H-6).

Further elution after evaporation of the solvent, followed by crystallization from EtOH–H_2O afforded **10**, mp 139—140°. Yield was 430 mg (10.4%).

5-Methylaminomethyl-2-thiouridine (10)——A solution of **9** (300 mg) in 10% AcOH (6 ml) was refluxed for 2 hr and evaporated to dryness. The residue was crystallized from EtOH–H_2O to afford **10**, mp 139—140°. Yield was 210 mg (77%). *Anal.* Calcd. for $C_{11}H_{17}O_5N_3S \cdot 1/2H_2O$: C, 42.30; H, 5.77; N, 13.46; S, 10.25. Found: C, 42.28; H, 5.86; N, 13.20; S, 10.21. UV λ_{max} nm (ε): 219.5 (14700), 273.5 (13700) at pH 2; 222 (12900), 239 (11200), 275.5 (15200) at pH 7.5; 241.5 (21100), 270.5 (15700) at pH 12; 220 (15200), 275 (14700) at pH 7.0.

5-Hydroxymethyl-2-thiouridine (11)——A solution of **2b** (660 mg) in 20% AcOH (20 ml) was heated in a boiling water bath and then evaporated to dryness. The residue was crystallized from MeOH to afford **11**, mp 192° (decomp.). Yield was 540 mg (93%). *Anal.* Calcd. for $C_{10}H_{14}O_6N_2S$: C, 41.38; H, 4.86; N, 9.65; S, 11.03. Found: C, 41.51; H, 4.92; N, 9.44; S, 10.98. UV λ_{max} nm (ε): 220 (15900), 278.5 (15900) at pH 2; 221 (15900), 277 (16800) at pH 7.5; 241 (21600), 272 (15400) at pH 12.

5-(N-Methyl benzamido)methyl-2-thiouridine (12) (R=H)——To an aqueous solution (20 ml) of **9b**·1/2H_2O (312 mg), adjusted to pH 8 with 0.5N NaOH. There was then added a solution of benzoic anhydride (1.13 g) in DMF (5 ml). The solution was kept at pH 8 by addition of 0.5N NaOH. After the period of 3 hr, consumption of alkali ceased. The solution was then adjusted to pH 12 with 2N NaOH and allowed to stand for 2 hr at room temperature and neutrallized with 2N HCl. The reaction mixture was concentrated to dryness. The residue was triturated with MeOH. The MeOH extract was concentrated to *ca.* 5 ml and then applied to a column of silica gel (10 g) and the column was washed with $CHCl_3$–MeOH (7:1). Eluate containing **12** (R=H) was collected and evaporated to dryness. The residue was crystallized from EtOH to afford **12** (R=H), mp 115—117°. Yield was 285 mg (70%). *Anal.* Calcd. for $C_{18}H_{21}O_6N_3S$: C, 53.07; H, 5.20; N, 10.32; S, 7.86. Found: C, 53.11; H, 5.13; N, 10.22; S, 7.83. NMR (DMSO-d_6) δ: 2.90 (3H, s, N–CH_3), 4.01 (2H, s, –CH_2N–), 6.60 (1H, d, J=2 Hz, 1′-H), 7.5 (5H, s, aromatic), 8.14 (1H, s, H-6). UV λ_{max} nm: 220, 277 (pH 2); 220, 277 (pH 7.0), 241, 271 (pH 12). IR ν_{max}^{Nujol} cm^{-1}: 1660, 1600, 1575.