SYNTHESIS OF 4-THIOURIDINE DERIVATIVES

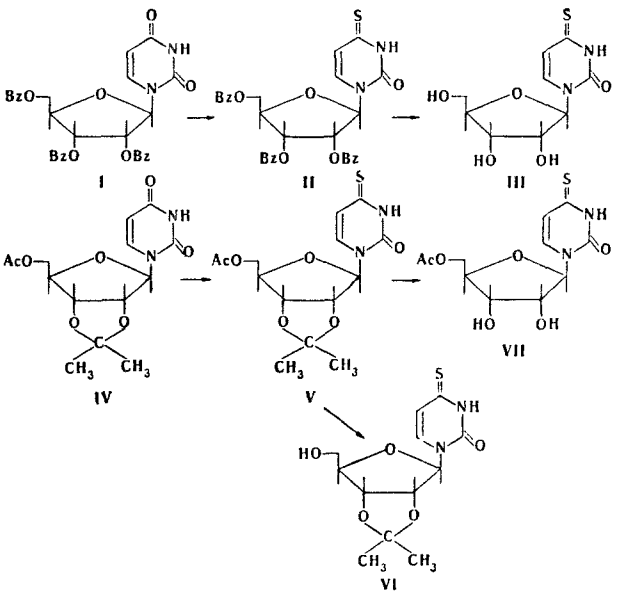
L. I. Kolobushkina, A. M. Kritsyn, and V. L. Florent'ev

UDC 547.963.32'854.4:542.945.32

The thionation of a protected uridine with the aid of P_2S_5 has given the corresponding 4-thiouridine derivatives, which have been phosphorylated at the 5' or the 2'(3')hydroxyl. On being heated in aqueous pyridine solution with liquid hydrogen sulfide, cytidine phosphates also form 4-thiouridine phosphates. 4-Thiouridine 2',3'-cyclophosphate has been obtained.

Among the minor components of some tRNAs have been found nucleotides containing sulfur in a pyrimidine or purine base [1-3]. In addition to this, nucleosides and nucleotides modified in the base are convenient instruments for the study of various enzymes. In view of this, it appears important and of interest to synthesize 4-thiouridine and its phosphate esters.

A very widespread method of introducing a thione group into position 4 of a pyrimidine nucleus of a nucleoside is thionation of the nucleoside with phosphorus pentasulfide [4-6]. This method has also been extended to nucleotides [7]. However, a defect of direct thionation is the necessity for protecting the sugar part of the molecule of the nucleoside. An ethanolic solution of sodium hydrogen sulfide has been used for the thionation of 2',4-di-O-methyluridine [8], but the preparation of the initial compound in this synthesis is again associated with the necessity for protecting the ribose hydroxyls. An easy replacement of the amino group in some nucleo-bases by a thione group with the aid of liquid hydrogen sulfide in aqueous pyridine solutions has recently been reported [9].



Institute of Molecular Biology, Academy of Sciences of the USSR, Moscow. Translated from Khimiya Geterotsiklicheskikh Soedinenii, No. 7, pp. 996-1000, July, 1973. Original article submitted May 29, 1972.

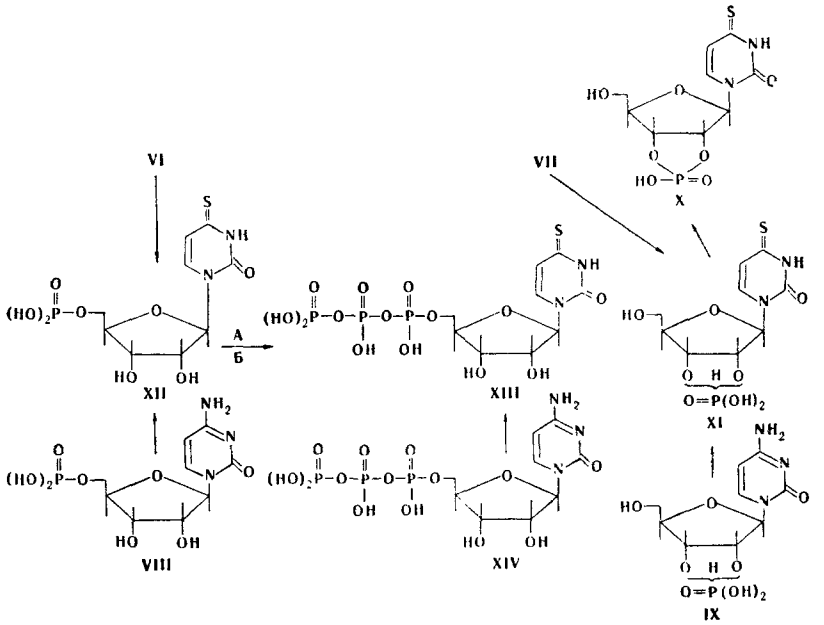
The present paper is devoted to a comparative study of various methods of obtaining 4-thiouridine and its derivatives. According to the first method (see [4, 5]), benzoylated uridine (I) was thionated with phosphorus pentasulfide in boiling pyridine. The thio derivative (II) obtained was debenzoylated with a methanolic solution of sodium methoxide, and the thiouridine (III) was phosphorylated by Scheit's method [10].

In the second case, acid- and alkali-labile protective groups for the hydroxy group of the ribose part of the molecule of the nucleoside were used. In this way, unambiguous phosphorylation was subsequently achieved.

After treatment with 2,2-diethoxypropane in acetone in the presence of acid catalysts (p-toluenesulfonic acid), uridine gave 2',3'-O-isopropylideneuridine. The latter was acetylated with acetic anhydride in pyridine. On thionation with phosphorus pentasulfide in pyridine [11], the 5'-O-acetyl-2',3'-O-isopropylideneuridine (IV) gave the protected thio compound (V). The acetyl protection was eliminated by treating (V) with aqueous ammonia in dioxane, and compound (VI) was obtained with a yield of 53%. Heating (V) in 50% acetic acid gave an 88% yield of (VII). An extremely convenient method for the purification of compounds (III and V-VII) is chromatography on columns of silica.

The thio derivatives of uridine (VI) and (VII) were phosphorylated by Scheit's method with β-cyanoethyl phosphate, using N,N'-dicyclohexylcarbodiimide as condensing agent.

Good results are given by the thionation of cytidine derivatives with liquid hydrogen sulfide in aqueous pyridine solution [9]. By this method, cytidine itself, cytidine 5'-phosphate (VIII) and cytidine 2'(3')-phosphate (IX) were converted into the corresponding derivatives of 4-thiouridine.



4-Thiouridine 2',3'-cyclophosphate (X) was obtained in good yield from 4-thiouridine 2'(3')-phosphate (XI) in the presence of dicyclohexylcarbodiimide in dimethylformamide solution.

We obtained the 4-thiouridine 5'-triphosphate (XIII) necessary for study in various enzyme systems by three methods. If cytidine 5'-triphosphate (XIV) is treated with liquid hydrogen sulfide in aqueous pyridine solution, under the reaction conditions the triphosphate undergoes degradation and the yield of the required product is negligible. The other two methods consist in the reaction of 4-thiouridine 5'-phosphate (XII) with di(tert-butylammonium) pyrophosphate in the presence of carbonyldiimidazole or sulfinyldiimidazole as condensing agent. The use of sulfinyldiimidazole is preferable – it is convenient to handle, the reaction with it is conpleted more rapidly, and the yield of triphosphate is higher.

EXPERIMENTAL

The UV spectra were taken on a Specord UV-VIS spectrophotometer and the PMR spectra on a Hitachi R-20A spectrometer with a working frequency of 60 MHz.

4-Thiouridine (III) was obtained by a published method [5]. Yield 41%, mp 136-137°C. UV spectrum (pH 7): λ_{max} 331 nm (ε 22,600). According to the literature [5], mp 135-138°C.

5'-O-Acetyl-2',3'-O-isopropylideneuridine (IV). To a solution of 4 g (14 mmoles) of 2',3'-O-isopropylideneuridine in 30 ml of absolute pyridine was added 3.3 ml of freshly distilled acetic anhydride, and the mixture was left at room temperature for 20 h. Then 15 ml of absolute methanol was added and the resulting mixture was kept for 1 h 30 min and was evaporated to dryness. The reaction products were used for thionation without purification.

5'-O-Acetyl-2',3'-O-isopropylidene-4-thiouridine (V). To a stirred boiling suspension of (IV) (14 mmoles) and 7.9 g (28 mmoles) of phosphorus pentasulfide in 150 ml of absolute pyridine was added 1.4 ml of water dropwise and very slowly (5 h), and the resulting mixture was left overnight. Then it was evaporated to dryness and the residue was treated with 100 g of crushed ice and after 1 h 30 min it was extracted with chloroform. The extract was dried with magnesium sulfate and evaporated, and the residue was dissolved in the minimum volume of chloroform and chromatographed on a column of silica (200 g) with elution by chloroform. Yield 4.2 g (87%), mp 143-144°C (from isopropanol) [11]. UV spectrum (pH 7): λ_{max} 329 nm (ε 18,700). PMR spectrum* (CCl_4), δ, ppm: 7.50 (d, $J_{5,6}$ = 14 Hz, 6-H); 7.38 (d, $J_{5,6}$ = 14 Hz, 5-H); 6.58 (s, 1'-H); 6.08 (m, 2'-, 3'-, and 4'-H); 5.35 (s, 5'-CH_2); and 3.17, 2.68, and 2,48 (three s, CH_3).

2',3'-O-Isopropylidene-4-thiouridine (VI). A solution of 1.7 g (5 mmoles) of (V) in 50 ml of a mixture of 12% aqueous ammonia and dioxane (1:1) was stirred at room temperature for 16 h. Then the reaction mixture was evaporated and traces of water were eliminated by distillation with ethanol and benzene, and the residue was recrystallized from isopropanol. Yield 0.8 g (53%), mp 181-182°C (according to the literature [11], mp 184°C). UV spectrum (pH 7): λ_{max} 330 nm (ε 21,000).

5'-O-Acetyl-4-thiouridine (VII). A solution of 0.1 g (0.3 mmole) of (V) in 30 ml of 50% acetic acid was heated at 70°C for 2 h. The solution was evaporated to dryness and was reevaporated with water and ethanol, and the residue was dissolved in a mixture of chloroform and methanol. This solution was deposited on a column containing 50 ml of silica, the column was washed with chloroform, and chromatography was performed with 5% of methanol in chloroform. The fractions containing the (VII) were combined and evaporated in dryness. Yield 0.08 g (88%), mp 143-145°C (from isopropanol). UV spectrum (in methanol): λ_{max} 332 nm (ε 19,000). PMR spectrum (in D_2O), δ, ppm: 7.61 (d, $J_{5,6}$ = 6 Hz, 6-H); 6.56 (d, $J_{5,6}$ = 6 Hz, 5-H); 5.85 (d, $J_{1',2'}$ = 6 Hz, 1'-H); 4.37 (d, 5'-CH_2); 4.33 (m, 2'-, 3'-, and 4'-H); 2.18 (s, CH_3CO).

4-Thiouridine 5'-Phosphate (XII). A. A mixture of 100 mg (0.33 mmole) of 2',3'-O-isopropylidene-4-thiouridine and 1 ml of a 1 M solution of β-cyanoethyl phosphate was dried by distillation with absolute pyridine (3 × 10 ml), the residue was dissolved in 10 ml of absolute pyridine, 0.68 g (3.3 mmoles) of dicyclohexylcarbodiimide was added, and the mixture was stirred at room temperature without the access of atmospheric moisture for 2.5 days. Then 20 ml of water was added, and after stirring for 1 h the precipitate was filtered off and was washed with 20 ml of 50% pyridine. The filtrate and the wash waters were combined, extracted with cyclohexane (2 × 20 ml), and evaporated. The residue was evaporated with water (3 × 10 ml). The residual oil was dissolved in 20 ml of 50% acetic acid and the mixture was heated at 100°C for 45 min and was then evaporated with water (3 × 10 ml). The residue was dissolved in 10 ml of 1 N KOH and the solution was heated at 100°C for 15 min. After cooling, it was filtered and the filtrate was passed through a column containing 50 ml of Dowex-50 resin (H^+ form), the eluent being collected in 10 ml of dilute (1:10) ammonia. The solution was evaporated to half-volume and deposited on a column containing 250 ml of DEAE-cellulose equilibrated with 0.05 M NH_4HCO_3. Chromatography was carried out with NH_4HCO_3 (linear gradient from 0.05 to 0.3 M; total volume 5 liters). The fractions containing the (XII) were combined, evaporated to small volume, and freeze-dried. Yield 55 mg (46%).

B. A solution of 0.4 g (1.1 mmole) of cytidine 5'-(disodium phosphate) in 5 ml of water, 5 ml of pyridine, and 10 ml of liquid hydrogen sulfide was heated in an autoclave at 60°C for 45 h. The reaction mixture was evaporated, the residue was treated with water, and the mixture was filtered. The filtrate was evaporated to small volume and the 4-thiouridine 5'-(disodium phosphate) was precipitated by the addition of an excess of absolute ethanol. Yield 0.3 g (71%). UV spectra: 0.1 N HCl – λ_{max} 333 nm (ε 22,300); pH 7 – λ_{max} 333 nm (ε 21,900); 0.1 N KOH – λ_{max} 319 nm (ε 18,700) (see [9]). PMR spectrum (in D_2O), δ, ppm: 7.98 (d, $J_{5,6}$ = 12 Hz, 6-H); 6.69 (d, $J_{5,6}$ = 12 Hz, 5-H); 5.97 (d, $J_{1',2'}$ = 3.8 Hz, 1'-H); 4.38 (s, 5-CH_2); and 4.43, 4.28, and 4.00 (m, 2'-, 3'-, and 4'-H).

*Abbreviations here and below: s) singlet; d) doublet; m) multiplet.

4-Thiouridine 2'(3')-phosphate (XI) was obtained by method A described for (XII) from 100 mg (0.33 mmoles) of (VII), 1 ml of a 1 M solution of β-cyanoethyl phosphate, and 0.68 g of dicyclohexylcarbodiimide with the omission of the stage of the hydrolysis by acetic acid. Yield 42.6 mg (36%).

B. A solution of 0.5 g (1.4 mmole) of the sodium salt of cytidine 2'(3')-phosphate in 5 ml of water, 5 ml of pyridine, and 10 ml of liquid hydrogen sulfide was heated in an autoclave at 60°C for 41 h. Then the reaction mixture was evaporated, the residue was treated with water, and the mixture was filtered. The filtrate was evaporated to small volume, and an excess of absolute ethanol was added. This gave 0.52 g (96%) of the sodium salt of 4-thiouridine 2'(3')-phosphate. UV spectra: 0.1 N HCl – λ_{max} 330 nm (ε 19,800); pH 7 – λ_{max} 330 nm (ε 20,000); 0.1 N KOH – λ_{max} 317 nm (ε 17,900) (see [6]). PMR spectrum (in D_2O), δ, ppm: 7.78, 7.70 (two d, $J_{5\,6}$ = 10 Hz, 6-H); 6.54 (d, $J_{5,6}$ = 10 Hz, 5-H); and 5.95 (two d, $J_{1',2'}$ = 6 Hz for the 2'-phosphate and 3 Hz for the 3'-phosphate).

4-Thiouridine 2',3'-Cyclophosphate (X). Freshly prepared 4-thiouridine 2'(3')-phosphate (from 240 mg of the disodium salt) was dried by distillation with absolute DMFA and was then dissolved in 20 ml of DMFA, and 800 mg of dicyclohexylcarbodiimide was added. The solution was stirred at room temperature without the access of atmospheric moisture. After 30 min, the precipitate that had deposited was filtered off and washed with water. The pH was brought to 7.5 by the addition of 0.1 N caustic soda and the mixture was evaporated. The residue was dried by evaporation with absolute pyridine and was then dissolved in the minimum volume of pyridine, and the solution was treated with an excess of ether. The resulting crystals were filtered off and washed with ether. Yield 175 mg (90%). UV spectra: 0.1 N HCl – λ_{max} 330 nm (ε 20,800); pH 7 – λ_{max} 330 nm (ε 20,600); 0.1 N KOH – λ_{max} 318 nm (ε 18,800). PMR spectrum (in D_2O), δ, ppm: 7.60 (d, $J_{5\,6}$ = 10 Hz, 6-H); 6.58 (d, $J_{5,6}$ = 10 Hz, 5-H); and 5.95 (d, $J_{1',2'}$ = 3 Hz, 1'-H).

4-Thiouridine 5'-Triphosphate (XIII). A. The ammonium salt of 4-thiouridine 5'-phosphate (50 mg; 0.14 mmole) was passed through a column containing 50 ml of Dowex-50 resin (pyridinium form) and was eluted with water (50 ml). The eluent was evaporated to dryness. The residue was dissolved in 4 ml of 50% pyridine, 0.034 ml of tributylamine was added, and the mixture was evaporated and was reevaporated with absolute pyridine and then with absolute DMFA. The residue was dissolved in 2 ml of DMFA, 69.7 mg (0.43 mmole) of carbonyldiimidazole was added, and the mixture was stirred for 2 h. Then 0.57 mmole of methanol was added and stirring was continued for another 30 min. After this, a solution of 0.7 mmole of tributylammonium pyrophosphate in 7.15 ml of DMFA was added to the reaction mixture and it was stirred at room temperature for 20 h. The precipitate was filtered off and was washed with DMFA and methanol, the filtrate was evaporated to dryness, the resulting residue was dissolved in 10 ml of water, the solution was filtered, the filtrate was diluted to 100 ml with a 0.1 M solution of NH_4HCO_3 and was deposited on a column of DEAE-cellulose (500 ml) and eluted with NH_4HCO_3 (linear gradient from 0.15 to 0.35 M, total volume 5 liters). This gave 41.7 mg (53%) of the triphosphate (XIII).

B. A solution of 0.14 mmole of the tributylammonium salt of 4-thiouridine 5'-phosphate, obtained as described above, in 1 ml of DMFA was mixed at 20°C with a solution of 0.14 mmole of sulfinyldiimidazole (from 144 mg of imidazole and 0.038 ml of thionyl chloride in 4 ml of tetrahydrofuran) in 1 ml of DMFA. The mixture was stirred at room temperature for 1 h, and then 0.57 mmole of methanol and 0.57 mmole of a solution of tributylammonium pyrophosphate in 4 ml of DMFA were added. The reaction mixture was stirred at room temperature for 3 h, the precipitate was filtered off and washed with a small volume of DMFA, the filtrate was evaporated, the residue was dissolved in a minimum amount of water, and the filtered solution was diluted to 100 ml with 0.1 M NH_4HCO_3 solution and deposited on a column containing DEAE-cellulose and was chromatographed with NH_4HCO_3 (linear gradient from 0.15 to 0.35 M, total volume 5 liters). This gave 52.7 mg of (XIII) (67%). UV spectrum (in water): λ_{max} 333 nm (ε 21,000).

Thionation of Cytidine 5'-Triphosphate. A solution of 0.5 g (0.8 mmole) of cytidine 5'-triphosphate in 3.3 ml of pyridine, 6.6 ml of water, and 6.6 ml of liquid hydrogen sulfide was heated at 60°C for 40 h. After evaporation, the residue was treated with water, the solution was filtered and made up to 100 ml of with 0.1 M NH_4HCO_3 solution and was then deposited on a column of DEAE-cellulose and was chromatographed with NH_4HCO_3 (linear gradient from 0.15 to 0.35 M; total volume 7 liters). The yield of 4-thiouridine monophosphate was 42%, of the diphosphate 46%, and of the triphosphate 12% (by UV spectroscopy).

The authors thank M. Ya. Karpeiskii for his constant interest in the work and for valuable critical observations.

LITERATURE CITED

1. M. N. Lipsett, J. Biol. Chem., 240, 3975 (1965).

2. M. N. Lipsett and B. P. Doctor, J. Biol. Chem., 242, 4072 (1967).
3. M. N. Lipsett, J. Biol. Chem., 242, 4067 (1967).
4. J. J. Fox, D. Van Praag, I. Wempen, I. L. Doerr, L. Cheong, J. E. Knoll, M. N. Eidinoff, A. Bendich, and G. B. Brown, J. Amer. Chem. Soc., 81, 178 (1959).
5. N. K. Kochetkov, É. I. Budovskii, V. N. Shibaev, and M. A. Grachev, Izv. Akad. Nauk SSSR, Ser. Khim., 1592 (1963).
6. M. Saneyoshi and F. Sawada, Chem. Pharm. Bull. (Tokyo), 17, 181 (1969).
7. M. Saneyoshi, Chem. Pharm. Bull. (Toyko), 19, 493 (1971).
8. M. J. Robins and S. R. Naik, Biochemistry, 10, 3591 (1971).
9. T. Ueda, M. Imazawa, K. Miura, R. Iwata, and K. Odajima, Tetrah. Lett., 2507 (1971).
10. G. M. Tener, J. Amer. Chem. Soc., 83, 159 (1961).
11. K.-H. Scheit, Ber., 101, 1141 (1968).