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A New Protecting Group. IV.[1] Syntheses of Several (2-Picolyl 1-oxide)thio Derivatives by Use of a New Thiol Group Reagent: 2-Picolylchloride 1-Oxide

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Alkylation of the thiols, *p*-thiocresol, 4-thiouridine, 4-thiouracil, 2-mercaptoinosine, cysteine, and glutathione with 2-picolyl chloride 1-oxide (**15**) afforded the corresponding 2-picolyl 1-oxide derivatives (**6**—**14**) in fair to good yields even in aqueous solution. Especially, this new thiol reagent was found to work well with 4-thiouridine and glutathione. 1-Oxido-2-picolylthio-derivative (**8**) was subjected to deblocking of the protecting group. Recovery of 2',3'-O-isopropylidene 4-thiouridine (**19**) after deblocking was found to be quite satisfactory (79.2% yield).

A number of naturally-occurring and synthetic thiol and thione derivatives of biological importance, such as cysteine, glutathione, pantetheine,[3] 4-thiouridine,[4] 6-mercaptopurine[5] and its β-D-ribofuranoside[6] have been described. 2-Aminoethanethiol has been reported to be protective against radiation injury in experimental animals.[7]

Thiol groups also paly a vital role in the functions of a large number of biopolymers. The active sites of enzymes or interactions of subunits may involve thiol groups. For this reason, numerous attempts have been made to develop specific thiol group reagents.[8] Such reagents are useful in determining whether the thiol group is crucial for the functioning of the biopolymer.[9]

For the syntheses of the thiol derivatives, there also occurs the need of protection of the thiol group against alkylation, acylation and oxidation. Benzyl, substituted benzyl, trityl, acyl and cyano groups have been employed for the protection of the thiol group.[10] Thus, synthesis of cysteine-containing peptides has been effected with the aid of benzyl,[11] tetrahydropyranyl,[12] and isopropylidene protecting group.[13] We may encounter a similar problem

1) Part III: K. Ikeda, K. Tsuchida, T. Monma and Y. Mizuno, *J. Heterocyclic Chem.*, **11**, 321 (1974).
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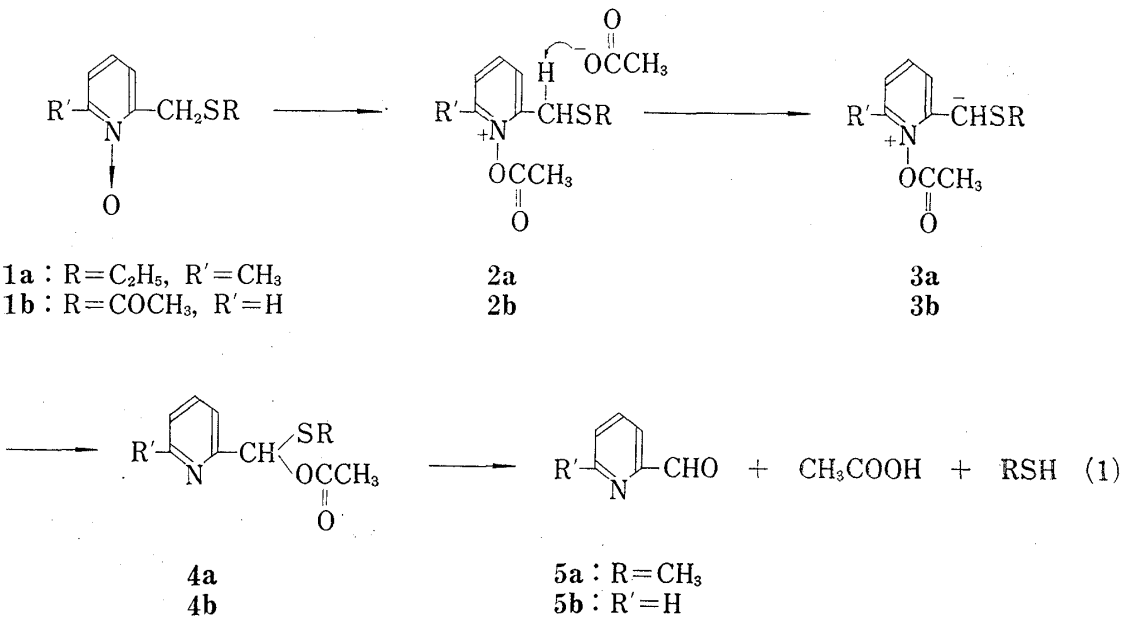
3) *a*) R.J. Williams, *J. Biol. Chem.*, **177**, 933 (1949); *b*) R. Schwyzer, *Helv. Chim. Acta*, **35**, 1903 (1952); *c*) J. Baddiley and E.M. Thain, *J. Chem. Soc.*, **1952**, 800; *d*) T.E. King, C.J. Stewart and V.H. Cheldelin, *J. Am. Chem. Soc.*, **75**, 1290 (1953); *e*) E. Walton, A.N. Wilson, F.W. Holly and K. Folkers, *ibid.*, **76**, 1146 (1954).
4) *a*) M.N. Lipsett, *J. Biol. Chem.*, **240**, 3975 (1965); *b*) J.J. Fox, D. Van Praag, I. Wempen, I.L. Doerr, L. Chenong, J.E. Knoll, M.L. Edinoff, A. Bendich and G.B. Brown, *J. Am. Chem. Soc.*, **81**, 178 (1959).
5) G.B. Elion, E. Burgi and G.H. Hitching, *J. Am. Chem. Soc.*, **74**, 411 (1952).
6) J.J. Fox, I. Wempen, A. Hampton and I.L. Doerr, *J. Am. Chem. Soc.*, **80**, 1669 (1958).
7) H. Langedorff and R. Koch, *Strahl Entherapy*, **99**, 567 (1956).
8) N. Kornblum and A. Acott, *J. Am. Chem. Soc.*, **96**, 590 (1974).
9) *a*) Y. Degami, H. Neumann and A. Patchornik, *J. Am. Chem. Soc.*, **92**, 6969 (1970); *b*) J.F.W. McOmie, "Advances in Organic Chemistry," Vol. 3, ed. by R.A. Raphael, E.C. Taylor and H. Wynberg, Interscience Publishers, New York, 1963, p. 191.
10) R.G. Hiskey, V.R. Rao and W.G. Rhodes in "Protecting Groups in Organic Chemistry," ed. by J.F.W. McOmie, Plenum Press, New York, 1973, pp. 235—308.
11) V. du Vigneaud, C. Ressler, J.M. Swan, C.W. Roberts and P.G. Katsoyannis, *J. Am. Chem. Soc.*, **76**, 3115 (1954); M. Bodansky and V. du Vigneaud, *ibid.*, **81**, 2504 (1959).
12) *a*) G.F. Holland and L.A. Cohen, *J. Am. Chem. Soc.*, **80**, 1158 (1958); *b*) L.R. Fedor and B.S.R. Murty, *ibid.*, **95**, 8407 (1973).
13) J.C. Sheehan and D.H. Yang, *J. Am. Chem. Soc.*, **80**, 1158 (1958).

in cases where we want to prepare oligonucleotides containing 4-thiouridine and mercapto-purine-β-D-ribofuranosides. Thus, there is an urgent need for the blocking group suitable for the thiol protection in the synthesis of such oligonucleotides.

Kasuga and Taguchi have investigated the acetic anhydride rearrangement of a thiol derivative: ethyl (6-methyl-2-picolyl 1-oxide) sulfide.[14] They have found that the reaction of this derivative with acetic anhydride afforded ethylthio (6-methyl 2-pyridyl)acetoxymethane (**4a**) in excellent yield which in turn readily hydrolyzed to give ethanethiol and 2-formyl 6-methylpyridine (**5a**, Chart 1).[14]

We have also examined the reactions of some 2-picolyl 1-oxide derivatives with acetic anhydride where with 2-picolyl 1-oxide thiolacetate (**1b**) a remarkable enhanced reactivity due to the so-called pπ-dπ resonance[15] (see footnote 16) as well as formula **3a** or **3b**) was observed in this rearrangement.[1]



1a : R=C_2H_5, R′=CH_3
1b : R=$COCH_3$, R′=H

2a
2b

3a
3b

4a
4b

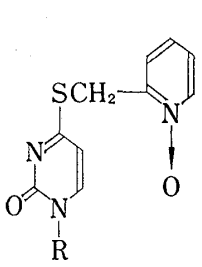
5a : R=CH_3
5b : R′=H

Chart 1

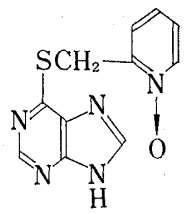
These informations coupled with the well-documented high degree of nucleophilicity exhibited by bivalent sulfur[17,18] prompted us to try 2-picolyl chloride 1-oxide as a removable thiol group reagent.

Thiols selected for the present investigation were *p*-thiocresol, 4-thiouracil, 2′,3′-O-isopropylidene-4-thiouridine, 2-mercaptoinosine, cysteine, and glutathione. Introduction of 2-picolyl 1-oxide protection into these compounds could be achieved by a modification of Lewis and co-workers' procedure[19] or by treatment of the thiols with a slight excess of 2-picolyl chloride 1-oxide hydrochloride[20] in the presence of two equivalents of the base.[21] The thiols of pyrimidine and purine series may absorb the ultraviolet light at much longer

14) S. Kasuga and T. Taguchi, *Chem. Pharm. Bull.* (Tokyo), **13**, 233 (1965).
15) W.J. Brehm and T. Levenson, *J. Am. Chem. Soc.*, **76**, 5389 (1954).
16) The rate determining step of "acetic anhydride rearrangement" is the proton removal by acetate anion. Therefore, the rate in the rearrangement is closely associated with the acidity of the methylenic protons.
17) E.O. Edwards and R.G. Pearson, *J. Am. Chem. Soc.*, **84**, 16 (1962).
18) K. Wallenfels and C. Streffer, *Biochem. Z.*, **346**, 119 (1966).
19) L.R. Lewis, O.W. Noell, A.G. Beaman and R.K. Robins, *J. Med. Pharm. Chem.*, **5**, 607 (1962).
20) P.T. Sullivan, M. Kester and S.J. Norton, *J. Med. Chem.*, **11**, 1172 (1968).
21) 1-Oxido-2-picolylsulfide (**12**) could also be prepared by alkylation of *p*-thiocresol with 1-oxido-2-pyridyldiazomethane. This reaction, however, afforded 2-picolyl hydrazone 1-oxide and di-*p*-tolylsulfide in addition to **12** whose yield was therefore found to be unsatisfactory.



6 : R = H
7 : R = CH_3
8 : R = Rf Rf = 2′, 3′-O-isopropylidene-β-D-ribofuranosyl



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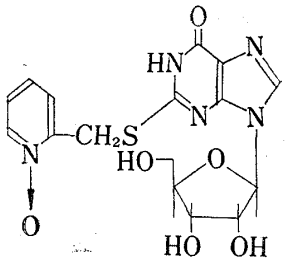

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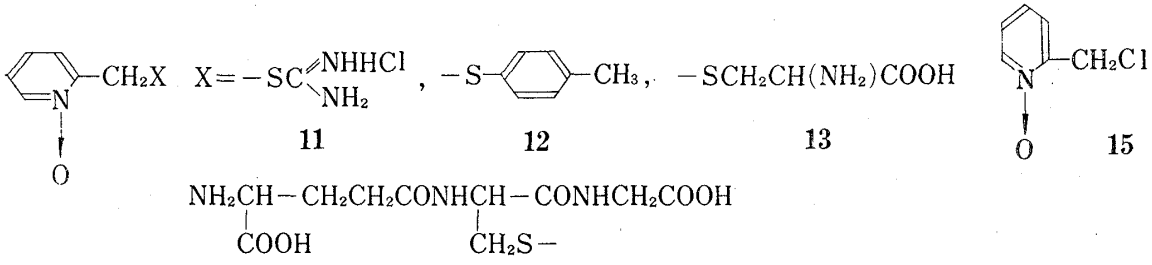


11 12 13 15

14

Chart 2

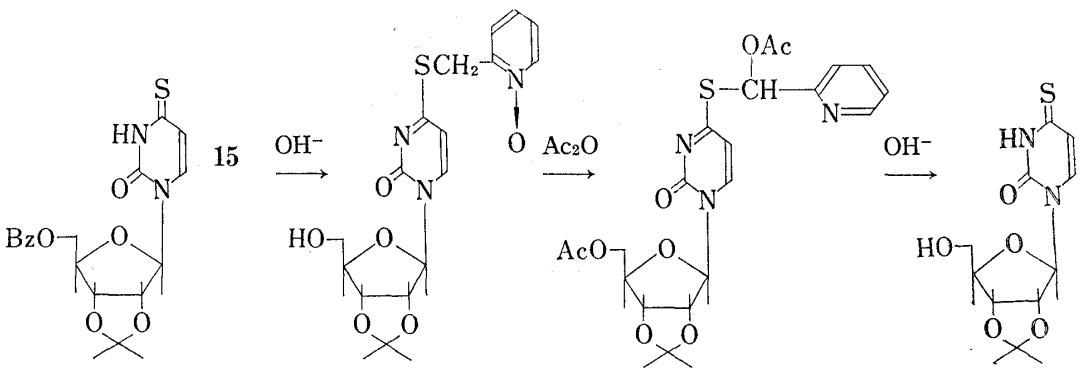
wavelength than the corresponding alkylthio-derivatives.[5] Therefore, in these cases the progress of the alkylation could be readily followed by examination of the ultraviolet absorption spectra of the reaction mixture.

The structure of each product was corroborated by the combustion values and the ultraviolet spectra.

It is to be noted that alkylation of thiols could be easily effected even in aqueous solution (see Experimental).

Conversion of **6** into **7** could be effected by application of a reported method.[22,23]

Although there would usually be no need to remove the blocking group introduced in the active site of enzymes, in cases of protection in the chemical synthesis the deblocking would be required. In order to demonstrate the removability of 1-oxido-2-picolyl protecting group, deblocking experiment was carried out with **8** as a representative.



16 17 18 19

Chart 3

22) H.L. Wheeler and T.B. Johnson, *J. Am. Chem. Soc.*, **42**, 30 (1909).
23) J.W. Jones and R.K. Johnson, *J. Am. Chem. Soc.*, **84**, 1914 (1962).

For deblocking, the substrate (**8**) was treated with acetic anhydride at room temperature for 3 hr. After mild alkaline hydrolysis, 2′,3′-O-isopropylidene-4-thiouridine was recovered in 79.2% yield.

Thus, in cases where conventional thiol blocking groups do not work well, *i.e.*, are not easily cleavable to give the parent thiol, this protection might be potentially quite useful. This new thiol group reagent has also an advantage of being able to be used in aqueous solution. This property is suitable for the modification of water soluble biopolymers.

In order for this reagent to be more useful, however, it is highly desirable to develop a fluoresent or spin-labelling 2-picolyl chloride analog. Investigation along this line is now under way in our laboratory.

Experimental[24]

4-(1-Oxido-2-picolylthio)-2-pyrimidinol (6)——4-Thiouracil (2.25 g, 17.5 mmoles) and 2-picolyl chloride 1-oxide hydrochloride (4.05 g, 22.5 mmoles)[20] was dissolved in 100 ml of MeOH containing 1 g of sodium. The solution was heated for 1 hr and filtered. The filtrate was concentrated to dryness. The residue was extracted with n-C_3H_7OH. Concentration of the extract afforded **6** (2.22 g, 54%). Recrystallization from 80% aqueous EtOH afforded an analytical sample, mp 208—210° (decomp.). *Rf* BuOH–H_2O (84: 16): 0.43; UV $\lambda_{max}^{H_2O}$ nm: 260, 298. *Anal.* Calcd. for $C_{10}H_9ON_3S$: C, 51.06; H, 3.85; N, 17.86; S, 13.61. Found: C, 50.89; H, 3.71; N, 17.75; S, 13.52.

4-(1-Oxido-2-picolylthio)-1-methyl-2-pyrimidone (7)——Compound (**6**, 935 mg, 3.97 mmoles), 0.57 g of methyl iodide and 220 mg of KOH were dissolved in 50% aqueous MeOH. The solution was refluxed for 2 hr. The reaction mixture was concentrated to dryness. The residue was extracted with $CHCl_3$. The solution was washed with 0.1N KOH solution and then with H_2O, dried over Na_2SO_4. The solid was filtered off. The filtrate was concentrated to dryness. The residue (430 mg) was crystallized from 80% aqueous EtOH. Yield, 230 mg, mp 188—190°. *Anal.* Calcd. for $C_{11}H_{11}O_2N_3S$: C, 53.01; H, 4.51; N, 16.85; S, 12.85. Found: C, 52.98; H, 4.43; N, 17.00; S, 12.81.

Deblocking of 7——1-Methyl-4-(1-oxido-2-picolylthio)-2(1H)pyrimidone (10 mg) was treated with 620 mg of acetic anhydride for 7 minutes at refluxing temperature. The cooled mixture was concentrated to dryness. The residue was free of acetic anhydride by three co-distillations with MeOH. The final residue was dissolved in 7.5 ml of 1N methanolic methoxide. The solution was kept at room temperature overnight. The mixture was neutralized with ion exchange resin (H^+ form). The filtrate was concentrated to dryness. UV λ_{min}^{pH7} nm: 244, 330; λ_{min}^{pH7} nm: 277. Chromatographic behavior of the product was found to be also identical with that of an authentic sample provided by Dr. T. Ueda, Hokkaido University.[23]

6-(1-Oxido-2-picolylthio)purine (9)——To a solution of 4.56 g (0.03 mmole) of 6-mercaptopurine in 60 ml of 1N NaOH was added in portions 5.4 g of picolyl chloride 1-oxide hydrochloride. After standing for 2 hr at room temperature, the solution was adjusted to pH 5 with AcOH. Crystals deposited were collected. Recrystallization from aqueous EtOH afforded **9**, yield, 6.20 g (79.8%), mp 230—231° (decomp.). UV $\lambda_{max}^{H_2O}$ nm: 260, 281 (sh), 288. *Anal.* Calcd. for $C_{11}H_9ON_5S$: C, 50.96; H, 3.47; N, 27.02; S, 14.19. Found: C, 50.91; H, 3.55; N, 27.00; S, 14.20.

2-(1-Oxido-2-picolylthio)inosine (10)——2-Mercaptoinosine (pyridinium salt, 6.54 g, 17.2 mmoles)[25] was dissolved in 40 ml of 1N NaOH and there was then added 3.1 g (17 mmoles) of picolyl chloride 1-oxide hydrochloride. The solution was kept at room temperature overnight, during which period cystals separated out. The crystals were collected, air-dried. Recrystallization from water afforded an analytical sample, mp 230—231° (decomp.), yield, 6.04 g (87.5%). UV $\lambda_{max}^{H_2O}$ nm: 258, 281 (sh). *Anal.* Calcd. for $C_{16}H_{17}O_6N_5S$: C, 47.14; H, 4.20; N, 17.20; S, 7.86. Found: C, 47.09; H, 4.22; N, 17.21; S, 7.85.

4-(1-Oxido-2-picolylthio)toluene (12)——To a solution of p-thiocresol (1.24 g, 0.01 mole) and 2-picolyl chloride 1-oxide hydrochloride (1.44 g, 0.01 mole) in 10 ml of EtOH was added 24 ml of 1N NaOH solution, and the solution was kept at room temperature for 12 hr and then concentrated to dryness. The residue

24) Ultraviolet spectra were determined on a Hitachi spectrophotometer Model 3T. Paper chromatography was carried out by the ascending technique on Toyo Roshi No 51A paper using 2-propanol–28% ammonium hydroxide–water (7: 2: 1). Silica gel for the thin-layer chromatography (TLC) refers to Kieselgel HF 254 (Merck). Solvent system: chloroform–ethanol (7: 1). The melting points are uncorrected. Elemental analyses were performed by a staff of the analytical Laboratory in Faculty of Pharmaceutical Sciences, Hokkaido University. Amino acid analysis was done on a Amino Acid Analyzer Hitachi KLA-3B. Resin, the first buffer and the second buffer employed were Hitachi No. 2612, 0.2M citrate buffer (pH 3.20) and 0.2M citrate buffer (pH 4.25), respectively.

25) This sample is a gift from Dr. Mikio Honjo, Takeda Pharmaceutical Co., Osaka. K. Imai, R. Marumo, K. Kobayashi, Y. Yoshida, J. Toda and M. Honjo, *Chem. Pharm. Bull.* (Tokyo), **19**, 576 (1971).

was crystallized from EtOAc or ether, mp 87—88°; yield, 823 mg (35.6%). UV λ_{max}^{EtOH} nm: 265. *Anal.* Calcd. for $C_{13}H_{13}ONS$: C, 67.53; H, 5.62; N, 6.06; S, 13.85. Found: C, 67.53; H, 5.70; N, 6.26; S, 13.74.

S-(1-Oxido-2-picolyl)thiouronium Hydrochloride (11)——A mixture of 2-picolyl chloride 1-oxide hydrochloride (2.16 g, 12 mmoles) and thiourea (890, 12 mmoles) in 12 ml of EtOH was heated for 1 hr. The resulting solution was kept at room temperature overnight, during which period voluminous precipitates were formed, and collected, crystallized from EtOH. Yield, 2.08 g (80%). UV λ_{max}^{EtOH} nm: 260. *Anal.* Calcd. for $C_7H_9N_3SHCl$: C, 38.18; H, 4.09; N, 19.09; S, 14.51; Cl, 16.13. Found: C, 38.12; H, 4.10; N, 19.13; S, 14.58; Cl, 16.33.

3-(1-Oxido-2-picolylthio)-alanine (13)——To a cooled solution (5°) of cysteine hydrochloride monohydrate (1.75 g, 10 mmoles) in 20 ml of 2N NaOH solution was added 2-picolyl chloride 1-oxide hydrochloride (1.97 g, 11 mmoles). The solution was kept at room temperature for 3.5 hr and then adjusted to pH 2.5 with a Dowex 50W resin (H^+ form). The solution was concentrated to a small volume (*ca.* 6 ml). Inorganic salt separated and filtered off. The filtrate was diluted to 30 ml with water and the solution was adjusted to pH 5.8 with an ion exchange resin (OH^- form). The resin was filtered off. The filtrate was concentrated to dryness. The residue was crystallized from MeOH–H_2O. Yield, 2.10 g (92%), mp 184—185° (decomp.). UV $\lambda_{max}^{H_2O}$ nm(ε): 258.5 (12000), $\lambda_{max}^{0.5N\ HCl}$ nm(ε): 260 (8700); NMR (D_2O) δ: 3.60 (2H, d, J=5.5 Hz, $SC\underline{H}_2CH(NH_2)COOH$); 4.42 (3H, t, J=5.5 Hz, $C\underline{H}(NH_2)COOH$ and H-6 of pyridine ring), 7.7—8.1 (3H, m, aromatic); 8.79 (1H, d, J=5.0, aromatic protons). Upon electrophoresis (1000 v.) in 0.05M triethylammonium bicarbonate, the product had a mobility of 10.7 cm compared to 13.8 cm for cysteine. *Anal.* Calcd. for $C_9H_{12}O_3N_2S$: C, 47.37; H, 5.30; N, 12.28; S, 14.02. Found: C, 47.32; H, 5.33; N, 12.10; S, 14.01.

S-(1-Oxido-2-picolyl)-glutathione (14)——To a cooled solution (0—5°) of glutathione (307 mg, 1 mmole) in 10 ml of 0.4N NaOH solution was added 2-picolyl chloride 1-oxide hydrochloride (180 mg, 1 mmole). The solution was kept at the same temperature for 5 hr. Product was purified by diethylaminoethyl-cellulose chromatography (column size: 3.5×40 cm). The column was packed and washed with a linear gradient of 0.25M triethyl ammonium bicarbonate and water. Fractions containing **14** were combined and concentrated to dryness. The residue was co-distilled several times with MeOH. The final residue was dissolved in 1 ml of water and the solution was adjusted to pH 5.0 and there was then added 1 ml of EtOH. The solution was kept in ice-box overnight, during which period crystals deposited and collected. Yield, 390 mg (94.2%). Amino acid analysis of the acid hydrolysate (hydrolysis conditions: 6N HCl, 110°, 24 hr) showed that the product (**14**) contained glutamic acid, 3-(1-oxido-2-picolylthio)alanine (**13**) and glycine in a ratio of 1.00: 0.95: 1.02.

2′,3′-O-Isopropylidene-S-(1-oxido-2-picolyl)-4-thiouridine (8)——A suspension of 5′-O-benzoyl-2′,3′-O-isopropylidene-4-thiouridine[26] (1.0 g, 2.48 mmoles) in 50 ml of 0.1N NaOH solution was stirred at room temperature until the clear solution resulted. It required *ca.* 1.5 hr. There was then added 530 mg (2.94 mmoles) of 2-picolyl chloride 1-oxide hydrochloride. The solution was stirred for 2 hr at room temperature. The neutralized solution was concentrated to dryness. The residue was triturated with chloroform. The chloroform solution was washed with water and dried over Na_2SO_4. The residue obtained after evaporation was crystallized from EtOH. Yield, 900 mg (88.2%), mp 156—157°. NMR (dimethylsulfoxide-d_6) δ: 1.35 (3H, s, isopropylidene), 1.55 (3H, s, isopropylidene), 3.70 (2H, d, J=4.0 Hz, H-5′), 4.69 (2H, s, CH_2S), 5.93 (1H, s, anomeric), 6.50 (1H, d, J=5.90 Hz, H-5), 7.5—7.8 (3H, m, aromatic), 8.17 (1H, d, J=5.9 Hz, H-6), 8.33 (1H, m, a proton of pyridine). UV $\lambda_{max}^{H_2O}$ nm(ε): 302 (12200); 261 (13500); λ_{max}^{1NHCl} nm(ε): 333 (sh. 7900), 314 (10100); 260 (8200). *Anal.* Calcd for $C_{18}H_{21}O_6N_3S$: C, 53.07; H, 5.20; N, 10.32; S, 7.86. Found: C, 52.88; H, 5.04; N, 10.21; S, 7.78.

Deblocking of 2′,3′-O-Isopropylidene-S-(1-oxido-2-picoyl)-4-thiouridine (8)——A suspension of 250 mg (0.614 mmoles) of 2′,3′-O-isopropylidene-S-(1-oxido-2-picolyl)-4-thiouridine (**8**) in 5 ml of acetic anhydride was stirred until the clear solution resulted. The solution was kept stirring for 1.5 hr. The solution was then concentrated to dryness. The residue was dissolved in $CHCl_3$ and applied to a silica gel column (column size: 1.5×30 cm). The column was washed with a mixture of $CHCl_3$–MeOH (9: 1). Eluate containing product was collected and concentrated to dryness (weight: 300 mg). NMR spectral data (the absence of a signal due to the methylene of SCH_2- in **8** and the presence of signals (2.15 ppm) due to acetoxymethine) indicated that rearrangement was complete. UV λ_{max}^{EtOH} nm: 304, 270.

A suspension of the rearranged product (**15**, 300 mg) in 5 ml of 0.05N NaOH was stirred at room temperature until the clear solution resulted. It required about 3 hr. The neutralized solution (with AcOH) was concentrated to dryness. The residue was crystallized from MeOH–AcOEt. Yield, 145 mg (79.2%), mp 172—173°. Mixed mp with an authentic sample[27] did not depress. Spectral data were also found to be identical with those of the authentic sample.

26) M. Ikehara, T. Ueda and K. Ikeda, *Chem. Pharm. Bull.* (Tokyo), **10**, 767 (1962).
27) M. Saneyoshi and F. Sawada, *Chem. Pharm. Bull.* (Tokyo), **17**, 181 (1967).