

antitumor activity, since we had already prepared a number of *N*-oxides of substituted pyridines, thiazole or imidazole derivatives, for our studies on protecting groups suitable for use in the synthesis of nucleosides, nucleotides, and oligonucleotides.⁴⁻⁶⁾

Compounds **5** through **10** are tosylhydrazones that we prepared for the present screening studies against sarcoma 180. Of these compounds, the first three were prepared according to the reported procedures.^{4,5)} The synthesis of compound **8** was carried out as shown in Chart 1.

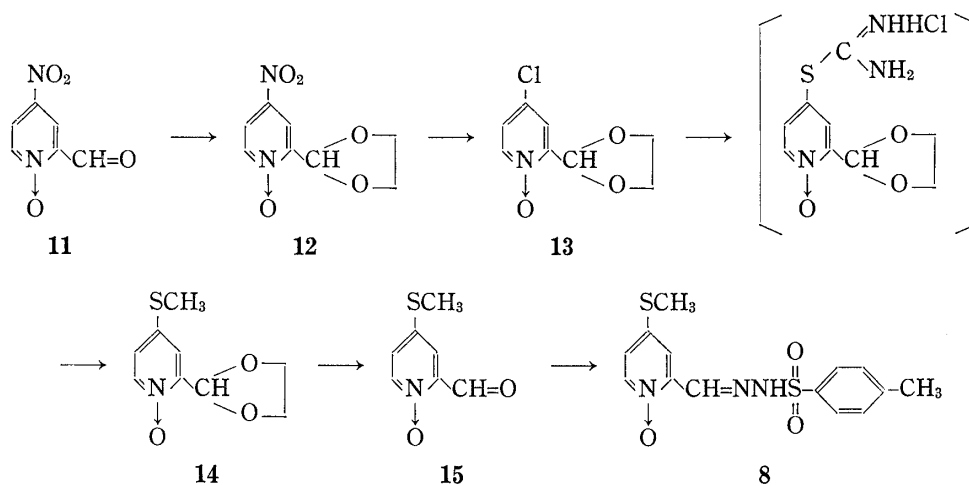


Chart 1

The most direct approach to 2-formyl-4-methylthiopyridine 1-oxide (**15**) appeared to be selenium dioxide oxidation of 4-methylthio-2-picoline 1-oxide. However, this approach did not work. Therefore, we adopted a new strategy, selecting readily accessible 2-formyl-4-nitropyridine 1-oxide (**11**)^{3b)} as the starting material. Treatment of **11** with ethylene glycol in the presence of a catalytic amount of *p*-toluenesulfonic acid in benzene on a Dean-Stark apparatus afforded the acetal (**12**) in good yield. Compound (**12**) was then treated with acetyl chloride at room temperature for one hour to give (**13**) in 82% yield. The reaction of the latter with thiourea gave the thiouronium salt, which in turn was converted to 2-formyl-4-methylthiopyridine ethylene acetal 1-oxide (**14**) with methyl iodide. Acidic hydrolysis of **14** afforded 2-formyl-4-methylthiopyridine 1-oxide (**15**). The yield of **15** was 31.6% on the basis of **13**. The reaction of **15** with *p*-tosylhydrazine gave rise to the corresponding *p*-tosylhydrazone (**8**). The *p*-tosylhydrazone (**7**) was similarly prepared from 2-formyl-4-nitropyridine 1-oxide.

Subsequently, preparation of *p*-tosylhydrazones of 2-formylazole 3-oxides was carried out. An attempt at selenium dioxide oxidation of 2-methylthiazole 3-oxide to provide 2-formylthiazoles was again unsuccessful. We next tried to prepare 2-acetoxymethyl-4-methylthiazole 3-oxide by *N*-oxidation of 2-acetoxymethyl-4-methylthiazole,⁷⁾ but obtained only an intractable mixture. Finally, we succeeded in the synthesis of 2-formyl-4-methylthiazole 3-oxide (**20**) by the alternate route formulated in Chart 2.

2,4-Dimethylthiazole-3-oxide (**16**)⁸⁾ was treated with excess (*ca.* 4-fold) benzaldehyde in the presence of ethanolic sodium ethoxide at ambient temperature to afford 4-methyl-2-styrylthiazole-3-oxide (**17**) in 60.2% yield. Its combustion values were found to be com-

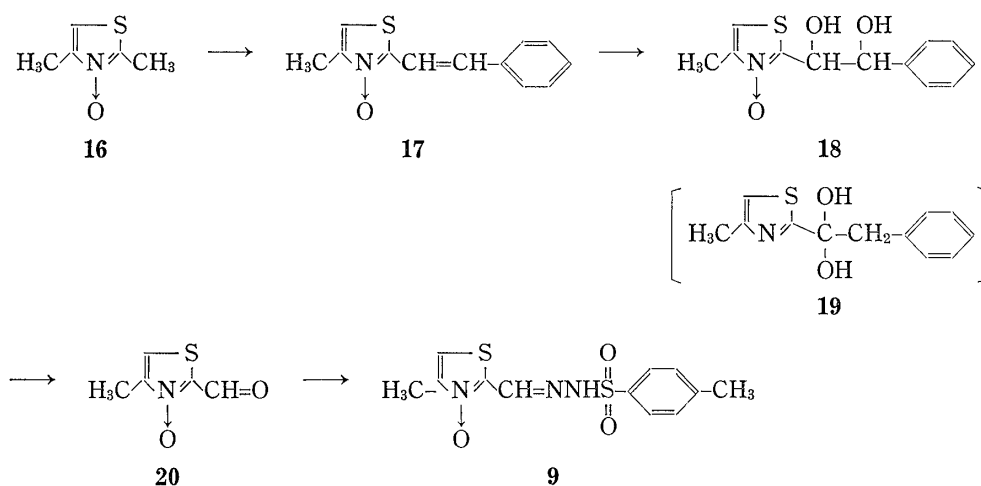
4) T. Endo, K. Ikeda, and Y. Mizuno, *J. Chem. Soc., Chem. Commun.*, **1973**, 673.

5) Y. Mizuno, T. Endo, and T. Nakamura, *J. Org. Chem.*, **40**, 1391 (1975).

6) Y. Mizuno, T. Endo, and K. Ikeda, *J. Org. Chem.*, **40**, 1385 (1975).

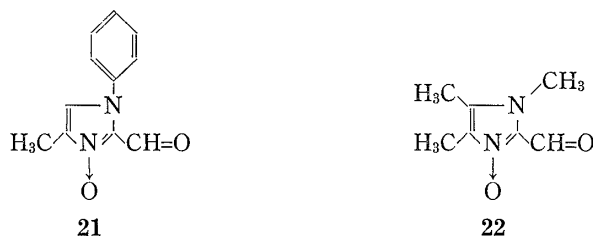
7) H.J. Anderson, D.J. Barnes, and Z.M. Khan, *Canada. J. Chem.*, **42**, 2375 (1964).

8) E. Ochiai and E. Hayashi, *Yakugaku Zasshi*, **67**, 34 (1947).



patible with the assigned structure. However, the product melted over the range of 96—133°, suggesting that it is a mixture of geometrical isomers. The mixture as such was, however, used for the subsequent step. On oxidation with osmium tetroxide in pyridine, **17** was consumed quite rapidly. However, the main product was not the desired 2-(1,2-dihydroxy-2-phenylethyl)-4-methylthiazole 3-oxide (**18**), but was presumably 4-methyl-2-thiazolyl benzyl ketone monohydrate (**19**); the structure of **19** has not yet been confirmed. The yield of **18** on isolation by TLC was only 8.6%. Its structure was supported by the results of analyses by mass spectrometry, IR, NMR, and combustion values. Oxidation of **18** with sodium metaperiodate gave rise to 2-formyl-4-methylthiazole-3-oxide (**20**) in 91.8% yield.

It is well established that direct oxidation of a 1-substituted imidazole does not afford the corresponding 3-oxide.⁹⁾ Accordingly, we have developed a novel method for the synthesis of 2-formyl-4-methyl-1-phenylimidazole-3-oxide (**21**).¹⁰⁾ Unfortunately, however, **21** reacted very sluggishly with *p*-tosylhydrazine, presumably because of steric hindrance of the neighboring bulky phenyl group, to give the corresponding *p*-tosylhydrazone in poor yield. Since our synthetic scheme was thus confronted with difficulty in the final stage, 2-formyl-1,4,5-trimethyl imidazole 3-oxide was prepared according to Ferguson and co-workers.¹¹⁾ The formyl derivatives (**20**) and (**22**) were converted to the corresponding *p*-tosylhydrazones (**9**) and (**10**) in a conventional way.



Antitumor activity was estimated by the method described in "Experimental".¹²⁾ The results obtained are summarized in Table I.

9) a) H. Stamm, "Organic N-Oxides," in F. Korte, *Methodium Chemicum*, **6**, 308 (1975); b) K. Schofield, M.R. Grimmett, and B.R.T. Keene, "The Azoles," Cambridge Univ. Press, London, 1976.

10) Y. Mizuno and Y. Inoue, *J. Chem. Soc., Chem. Commun.*, **1978**, 35.

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TABLE I. Antitumor Activity of the Present Compounds Against Ascites Sarcoma 180

Compound	Dose (mg/kg/day)	Tumor growth T/C%	Antitumor activity ^{a)}	Mortality (died/used)
1	300	—	Toxic	1/6
	100	0	‡‡	0/6
	30	60	+	0/6
5	300	—	Toxic	6/6
	100	0	‡‡	0/6
	30	47	+	0/6
6	300	—	Toxic	6/6
	100	26	‡‡	0/6
	30	60	+	0/6
7	300	—	Toxic	3/6
	100	15	‡‡	0/6
	30	44	+	0/6
8	100	—	Toxic	6/6
	30	39	‡‡	0/6
	10	112	—	0/6
9	30 ^{b)}	112	—	0/6
10	30 ^{b)}	102	—	0/6

a) —66 or more in T/C%; +65 —41; ‡‡40 —11; ‡‡‡ 10 —0.

b) The amount of compound available was limited, so the dose could not be increased.

p-Tosylhydrazones of the pyridine *N*-oxides series are quite effective against ascites sarcoma 180, whereas *p*-tosylhydrazones of the azole *N*-oxide series are less active or inactive.

Experimental

Determination of Antitumor Activity Against Ascites Sarcoma 180—Groups of six female ddN mice weighing 20 ± 2 g were implanted intraperitoneally (*i.p.*) with 1×10^7 cells of sarcoma 180. All compounds to be tested were suspended in 0.5% carboxymethylcellulose in 0.9% NaCl solution and injected *i.p.* at various doses once daily for 5 days, starting 24 hr after transplantation. Antitumor activity was evaluated in terms of the total packed cell volume (TPCV) ratio (T/C%) on the 7th day after implantation.¹²⁾

General—The melting points are uncorrected and were taken on a Yamato melting point apparatus. Infrared spectra were taken with a JASCO IRA-1 spectrometer. The NMR spectra were recorded on a Hitachi NMR spectrometer, model R24, and the following abbreviations are used: s, single, d, doublet, and bd, broad doublet. The chemical shifts are given in parts per million downfield from Me₄Si. The mass spectrometer employed was a Hitachi RMU-6E instrument, at an ionizing energy of 80 eV. Elemental analyses were performed by the staff of the Analytical Center of Hokkaido University.

2-Formyl-4-methoxypyridine-1-oxide *p*-Tosylhydrazone (6)—A pyridine (30 ml) solution of (4-methoxy-2-pyridyl 1-oxide)methanol (4.17 g, 26.9 mmol) and selenium dioxide (1.7 g, 15.3 mmol) was heated under reflux with vigorous stirring for 9 hr. The cooled solution was filtered and the filtrate was concentrated to dryness *in vacuo* to give 2-formyl-4-methoxypyridine-1-oxide *p*-tosylhydrazone; NMR (CDCl₃) δ : 3.89 (s, 3H, —OCH₃), 6.98 (q, $J_{5,6} = 7.2$ Hz, $J_{5,3} = 3.2$ Hz, 1H, H-5), 7.28 (d, $J_{3,5} = 3.2$ Hz, 1H, H-3), 8.10 (d, $J_{6,5} = 7.2$ Hz, 1H, H-6), 10.61 (s, 1H, —CHO). The residue was dissolved in methanol (40 ml) containing *p*-tosylhydrazine (7 g, 37.6 mmol). The precipitate was collected; yield, 4 g (46.3%). mp 108—109° (dec.), NMR (DMSO-*d*₆) δ : 2.34 (s, 3H, CH₃), 3.82 (s, 3H, OCH₃), *ca.* 7.1 (m, 2H, H-3 and H-5), 7.40 (d, $J = 7.2$ Hz, 1H, H-6), 8.40 (s, 1H, azomethine-proton). *Anal.* Calcd for C₁₄H₁₅N₃O₄S: C, 52.33; H, 4.67; N, 13.08; S, 9.97. Found: C, 52.74; H, 4.69; N, 12.81; S, 9.72.

2-Formyl-4-nitropyridine Ethylene Acetal 1-Oxide (12)—The hydrate form of 2-formyl-4-nitropyridine 1-oxide (11) was dehydrated by codistillation with dry benzene on a Dean-Stark apparatus. The corresponding acetal was then prepared in a way similar to that described for the preparation of 2-chloro-2-formylpyridine ethylene acetal.^{3c)} Crystallization from methanol gave an analytical sample, mp 145—146°. Yield, 75%. *Anal.* Calcd for C₈H₈N₂O₄: C, 48.98; H, 4.11, N, 14.28. Found: C, 48.99; H, 4.32; N, 14.44.

4-Chloro-2-formylpyridine Ethylene Acetal 1-Oxide (13)—Compound 12 (10 g, 49.7 mmol) was slowly added in portions to freshly distilled acetyl chloride (40 ml, 0.56 mol). The mixture was stirred for 1 hr at room temperature, during which period complete dissolution occurred. After confirming by TLC (silica gel; chloroform–ethanol 1:1) that the starting material had completely disappeared, the whole was poured into ice-water containing Na₂CO₃ (160 g). The product was extracted with chloroform. The solution was

concentrated to dryness *in vacuo*. Crude yield, 7.8 g (82%). To obtain an analytical sample, a portion of the product was recrystallized from ether, mp 106–108°. *Anal.* Calcd for $C_8H_8ClNO_3$: C, 47.66; H, 4.00; N, 6.95. Found: C, 47.63; H, 3.88, N, 6.94.

2-Formyl-4-methylthiopyridine Ethylene Acetal 1-Oxide (14)—Compound 13 (7.8 g, 38.7 mmol) and thiourea (2.94 g, 38.7 mmol) were dissolved in absolute ethanol (100 ml), and the solution was heated under reflux for 3 hr. On cooling, precipitates deposited. These were collected and dissolved in a mixture of H_2O (60 ml), ethanol (60 ml) and 10% sodium hydroxide solution (30 ml). Methyl iodide (5.6 g, 39.4 mmol) was added to the resulting solution and the whole was stirred at room temperature for 1 hr. The solution was then concentrated to dryness *in vacuo*. The residue was taken into water (*ca.* 200 ml). The aqueous solution was extracted with chloroform and the extract was successively washed with aqueous $Na_2S_2O_3$ (38.4 mmol) and water, and finally dried over Na_2SO_4 . Crude yield, 3.9 g (47.3% yield); mp of picrate, 115–117°. *Anal.* Calcd for $C_9H_{11}NO_3S \cdot C_6H_3N_3O_7$: C, 40.73; H, 3.19; N, 12.67. Found: C, 40.70; H, 3.19; N, 12.48.

***p*-Tosylhydrazone of 2-Formyl-4-methylthiopyridine 1-Oxide (8)**—2-Formyl-4-methylthiopyridine ethylene acetal 1-oxide (3.9 g, 18.3 mmol) was dissolved in 20% aqueous HCl (130 ml) and the solution was heated for 1.5 hr at 110–120° (oil bath). After confirming by TLC (solvent, chloroform: ethanol 10: 1) that hydrolysis was complete, the mixture was neutralized with solid Na_2CO_3 . The residue was treated with $CHCl_3$ (5 × 200 ml). Removal of the chloroform left a yellowish gum (2.88 g). The gum was dissolved in methanol (13 ml), and a methanol solution of *p*-toluenesulfonylhydrazine (4.76 g, 25.6 mmol, 1.4 equiv.) was added. The mixture was allowed to stand at room temperature overnight. Crystals which had deposited were collected and washed well with cold methanol. Yield, 1.95 g (31.6%); mp 117–119° (dec.). *Anal.* Calcd for $C_{14}H_{15}N_3O_3S_2$: C, 49.84; H, 4.48; N, 12.45; S, 19.01. Found: C, 49.75; H, 4.40; N, 12.31.

2,4-Dimethylthiazole—This compound was prepared according to a reported method¹³ with a slight modification. A chloroform solution (50 ml) of bromoacetone (72.5 g, 0.53 mol) was added dropwise to a cooled and stirred suspension of thioacetamide (39.7 g, 0.53 mol) in chloroform (400 ml). The mixture was stirred for 2 days at room temperature. The solid was then collected by suction and air-dried, the yield of 1-iminoethylthiopropanone hydrobromide being 90 g (80% yield). This was used for subsequent cyclization without further purification. However, the structure and purity were confirmed by NMR spectroscopy of a neutralized sample. NMR ($CDCl_3$) δ : 1.54 (s, 3H, CH_3), 2.19 (s, 3H, CH_3), 3.21 (d, $J=12$ Hz, 1H, gemH), 3.32 (d, $J=12$ Hz, 1H, gemH), 6.31 (s, 1H, =NH). The hydrobromide (35.5 g, 0.167 mol) was dissolved in acetic acid (150 ml), and Na_2CO_3 (17.7 g, 0.167 mol) was carefully added. The mixture was heated at 40–50° overnight. Water was then added. The resulting solution was carefully neutralized with conc. sodium hydroxide solution and 2,4-dimethylthiazole was extracted with chloroform. The residual oil obtained by removal of the solvent was distilled. The fraction boiling at 50–54°/47 mmHg was collected; yield, 12.6 g (66.8%). NMR ($CDCl_3$) δ : 2.37 (bs, 3H, 5- CH_3), 2.62 (s, 3H, 2- CH_3), 6.64 (bs, 1H, H-5).

2,4-Dimethylthiazole-3-oxide (16)—Compound 16 was prepared essentially according to a reported method.¹⁴ A solution of 2,4-dimethylthiazole (10.4 g, 92 mmol) in acetic acid (50 ml) was treated with 30% H_2O_2 (15.7 g, 138 mmol, 1.5 equiv.). The solution was heated at 80° for two days. An equal volume of water was added and the whole was concentrated to the original volume. This process was repeated three times. The final solution was dissolved in an equal volume of $CHCl_3$ -EtOH (1: 1 v/v) and neutralized with solid Na_2CO_3 . The product was purified by silica gel column chromatography. Yield, 10.0 g (84.2%); NMR ($CDCl_3$) δ : 2.59 (s, 3H, 2- CH_3), 2.38 (bs, 3H, 4- CH_3), 7.02 (bs, 1H, 5-H).

4-Methyl-2-styrylthiazole-3-oxide (17)—A solution of 2,4-dimethylthiazole-3-oxide (16) (1.6 g, 12.4 mmol) in ethanol (20 ml) was treated with ethanolic (20 ml) sodium ethoxide ($NaOC_2H_5$, 14.95 mmol). To the resulting (cooled and stirred) solution, benzaldehyde (5.5 g, 51.88 mmol) was added. Stirring was continued for 8 hr. Acetic acid (10 ml) was then added to the solution with stirring and the acidic solution was concentrated to dryness. The residue was taken up in water (20 ml) and the solution was treated with chloroform (3 × 30 ml), dried and filtered. The product was isolated by preparative TLC, mp 96–122–133°. Yield, 1.62 g (60.2%). NMR ($CDCl_3$) δ : 2.40 (s, 3H, CH_3); 6.67 (s, 2H, vinylic proton), around 8.00 (m, 6H, aromatic proton). MS: *m/e* 217 (M^+), 201 (M^+-16), 200 (M^+-17 , base peak). *Anal.* Calcd for $C_{12}H_{11}NO_2S$: C, 66.36; H, 5.07; N, 6.45; S, 14.74. Found: C, 66.48; H, 5.02; N, 6.46; S, 14.57.

2-(1,2-Dihydroxy-2-phenylethyl)-4-methylthiazole-3-oxide (18)—A pyridine solution (5 ml) of osmium tetroxide (1.03 g, 4.055 mmol) was added to a cooled solution of 4-methyl-2-styrylthiazole-3-oxide (17), (950 mg, 4.38 mmol) in pyridine (5 ml). Stirring was continued for 4 hr at room temperature (over this period, the solution turned red and insoluble material gradually deposited). The solution was then concentrated to dryness. The residue was taken up with $CHCl_3$ (80 ml) and this solution was treated with H_2S overnight. The solution was again concentrated to dryness, and the residue was dissolved in 5 ml of $CHCl_3$ /EtOH (1: 1). Insoluble material was filtered off. The filtrate, after concentration to a small volume, was applied to a silica gel column [silica gel 15 g, eluting with $CHCl_3$ /EtOH (1000: 3)]. The fast-moving fraction,

13) R.P. Kurkijy and F.V. Brown, *J. Am. Chem. Soc.*, **74**, 5778-9 (1952).

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afforded, after removal of the solvent, 195 mg of a homogeneous residue. The 4-methyl-2-thiazolyl benzyl ketone monohydrate structure (**19**) was tentatively deduced on the basis of spectral (IR, NMR, UV, and mass) and analytical data. *Anal.* Calcd for $C_{12}H_{13}NO_3S$: C, 61.28; H, 5.53; N, 5.96; S, 13.62. Found: C, 60.99; H, 5.51; N, 5.92; S, 13.39.

From the slowly moving band, 2-(1,2-dihydroxy-2-phenyl)-4-methylthiazole-3-oxide was obtained in 8.6% yield. NMR ($CDCl_3$) δ : 2.22 (s, 3H, 4- CH_3); 5.40 (d, $J=4$ Hz, $CHOH$), 5.40 (d, $J=4$ Hz, $CHOH$). UV λ_{max}^{MeOH} nm: 259 and 273; λ_{max}^{MeOH} nm: 250 and 265.

2-Formyl-4-methylthiazole-3-oxide (20)—Compound **18** (217 mg, 1 mmol) was dissolved in 14 ml of EtOH-H₂O (7:2 v/v), and an aqueous solution (4 ml) of sodium metaperiodate (214 mg, 1 mmol) was added. The solution was stirred for 1.5 hr, then the reaction mixture was filtered. The filtrate was concentrated to dryness. The residue was subjected to preparative TLC [chloroform/ethanol (20:2)]. Benzaldehyde (31.4 mg) was recovered from a fast-moving band. From the slower moving band, 2-formyl-4-methylthiazole-3-oxide was obtained in 91.8% yield. NMR ($CDCl_3$): 2.39 (s, 3H, CH_3), 7.41 (s, 1H, H-5), 10.22 (s, 1H, CHO). MS m/e : 143 (M^+); 127 (M^+-O); 126 (M^+-O-1); 115 (M^+-CO).

***p*-Tosylhydrazone (12)**—A solution of the aldehyde (8 mg) (containing a small amount of 4-methylthiazol-2-yl benzyl ketone) in methanol (1 ml) was treated with a methanol solution (1 ml) of *p*-tosylhydrazine (200 mg). The solution was allowed to stand at room temperature for 20 min. The precipitate which had deposited was collected. This material was recrystallized from $CH_3COOH-H_2O$, mp 134–135.5°. Yield, 40.7 mg. NMR ($DMSO-d_6$) δ : 2.17 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 7.40 (d, $J=9.6$ Hz, 2H), 7.76 (s, d, $J=9.6$ Hz, 3H), 8.23 (s, 1H, azomethinic proton). *Anal.* Calcd for $C_{12}H_{13}N_3O_3S$: C, 46.32; H, 4.18; N, 13.50; S, 20.57. Found: C, 46.19; H, 4.19; N, 13.37; S, 20.33.

1,4,5-Trimethylimidazole 3-Oxide *p*-Tosylhydrazone (10)—2-Formyl-1,4,5-trimethylimidazole 3-oxide (**22**) was prepared according to Ferguson and co-workers.¹¹ A methanol (2 ml) solution of *p*-tosylhydrazine (220 mg, 1.18 mmol) was added to an ethanol (2 ml) solution of **22** (91 mg, 0.59 mmol). The solution was stirred for 1 hr at room temperature. The reaction mixture was then concentrated to dryness. The residue was dissolved in 1 ml of acetic acid and then water was added until faint turbidity was seen. The whole was kept in a refrigerator overnight. Crystals which deposited were collected by suction, and air-dried. Yield, 91 mg (48%), mp 135–138°. *Anal.* Calcd for $C_{14}H_{18}N_4O_3S \cdot 3/2H_2O$: C, 50.29; H, 5.78; N, 16.76; S, 9.58. Found: C, 50.65; H, 5.80; N, 16.43; S, 9.28.

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