$J=4$ Hz), 7.25 (1H, doublet, $J=4$ Hz). Mass spectrum: $M^+=126$. *Anal.* Calcd. for $C_6H_6O_3$: C, 57.14; H, 4.80. Found: C, 56.40, H, 5.01.

Compound I Diacetate——Compound I (100 mg) was reacted with a mixture of acetic anhydride and pyridine under reflux for 2 hr and the reaction mixture was worked up as usual. IR ν_{max}^{neat} cm^{-1}: 1755, 1680, 1580. NMR (in $CDCl_3$) δ: 2.12 (6H, singlet), 4.68 (2H, singlet), 6.50 (1H, doublet, $J=4$ Hz), 7.25 (1H, doublet, $J=4$ Hz). *Anal.* Calcd. for $C_{10}H_{10}O_5$: C, 57.14; H, 4.80. Found: C, 56.69; H, 4.80.

Compound I 3,5-Dinitrobenzoate——Compound I (100 mg) was reacted with 3,5-dinitrobenzoyl chloride (150 mg) in pyridine (2.0 ml) at room temperature overnight. mp 122—123.5°. IR ν_{max}^{Nujol} cm^{-1}: 3050, 1740, 1680, 1540, 1350. NMR (in $CDCl_3$) δ: 3.52 (2H, singlet), 6.75 (1H, doublet, $J=4$ Hz), 7.25 (1H, doublet, $J=4$ Hz), 9.10 (3H, multiplet), 9.60 (1H, singlet). Mass spectrum *m/e*: 320, 290, 195, 179, 165, 149, 125, 109. *Anal.* Calcd. for $C_{13}H_8O_8N_2$: C, 48.76; H, 2.52; N, 9.47. Found: C, 48.52; H, 2.21; N, 8.75.

Properties of Compound II——mp 162—165°. UV λ_{max}^{EtOH} mμ (ε) 228 (18600), 235 (19500), 243 (12400). Mass spectrum *m/e*: 379, 378, 364, 204, 150. *Anal.* Calcd. for $C_{27}H_{41}N$: C, 85.42; H, 10.89; N, 3.69. Found: C, 85.53; H, 10.80; N, 3.90.

Properties of Compound III——mp 208—210°. Mass spectrum *m/e*: 397, 396, 382, 204, 150.

Comparisons of Compound III with Authentic Solanidine——In the following "a" means authentic and "s" means the isolated compound III. Thin-layer chromatography (silica plate): Solvent A ($CHCl_3$:EtOH $=10:1$). *Rf* a=0.51, s=0.49; Solvent B (C_6H_6:C_6H_5N:AcOH$=9:1:0.1$), *Rf* a=0.60, s=0.57. Paper partition chromatography (descend., Toyo Roshi No. 50 sprayed with 1N tartaric acid): solvent ($CHCl_3$: cellosolve acetate:$C_6H_5N=60:40:4$), developing time 4.5 hr, distance moved, a=13.5 cm, s=13.5 cm. IR (Nujol): superposable. Mixed mp: a=207—211°, s=208—210°, mixt.=207—211°.

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Synthetic Nucleosides and Nucleotides. VII. A Direct Replacement of 6-Thiol Group of 6-Thioinosine and 6-Thioguanosine with Hydrazine Hydrate1)

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As a conventional method for the synthesis of 6-substituted purine ribonucleosides have been employed the reaction of 6-halogeno or 6-alkylmercaptopurine ribonucleosides with several nucleophiles.3) But, because the 6-thiol group of purine nucleosides is known to be less reactive than the 4-thiol group of pyrimidine nucleosides, direct replacement at 6-position of 6-mercapto-9-β-D-ribofuranosylpurine (6-thioinosine) (Ia) and 2-amino-6-mercapto-9-β-D-ribofuranosylpurine (6-thioguanosine) (Ib) with amino or substitute amino group has not been reported and failed when attempted.4)

In previous paper of this series, the authors described the synthesis of 8-hydrazino derivatives of guanosine and xanthosine from the corresponding 8-bromo derivatives by treatment with 60% hydrazine hydrate in aqueous or methylcellosolve solution under mild condition.5)

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1) Part VI of this series: M. Saneyoshi and F. Sawada, *Chem. Pharm. Bull.* (Tokyo), **17**, 181 (1969).
2) Location: *a) Tsukiji 5-chome, Chuo-ku, Tokyo*; *b) Numakage, Urawa, Saitama.*
3) J.A. Montgomery and H.J. Thomas, *Advan. Carbohyd. Chem.*, **17**, 301 (1962).
4) T. Naito, K. Ueno, and F. Ishikawa, *Chem. Pharm. Bull.* (Tokyo), **12**, 951, (1964).
5) M. Saneyoshi, *Chem. Pharm. Bull.* (Tokyo), **16**, 1616 (1968).

In these experiments, direct amination of 8-bromo derivatives were attempted under several conditions but failed. Therefore, 8-hydrazinoguanosine or 8-hydrazinoxanthosine was refluxed with Raney Ni for 3 hr and the corresponding amino derivatives were obtained in a high yield.

This paper reports the direct replacement of thiol group of Ia and Ib with hydrazino group in similar manner as previously described, affording 6-hydrazino-9-β-D-ribofuranosylpurine (IIa) and 2-amino-6-hydrazino-9-β-D-ribofuranosylpurine (IIb).

TABLE I. Hydrazination of 6-Mercapto-9-β-D-ribofuranosylpurine at 90—100° for 2 hr on Various Hydrazine Concentration

Hydrazine concentration (%)	Yield of IIa (%)	Hydrazine concentration (%)	Yield of IIa (%)
95	30.5	40	54.0
80	47.5	20	42.7
60	80.0	10	recovered

First, hydrazination of Ia with hydrazine hydrate was studied, the results being summerized in Table I. For example, Ia was added to the solution of 60% hydrazine hydrate in methylcellosolve. After 2 hr's heating of the reaction mixture in a boiling water–bath, ultraviolet (UV) absorptionmaxima at 220 mμ and 317 mμ of the solution, which were characteristic of Ia, were completely disapeared and a new absorption maximum appeared at 264 mμ. When the solvent was removed under reduced pressure, white powder remained. This product was recrystallized from ethanol to give white fine micro–needles in 80% yield, which was identified with an authentic 6-hydrazino-9-β-D-ribofuranosylpurine[6] by mixed melting point and UV and infrared (IR) spectroscopic measurements.

This product (IIa) was reacted easily with sodium nitrite in acetic acid to give crystalline material (IIIa) which was identified with an authentic 3-β-D-ribofuranosyltetrazolo(5,4-*i*)-purine by mixed melting point, and UV and IR spectorophotometric determinations. Compound IIa was reduced by 3 hr's refluxing with Raney Ni to a crystalline product (IVa), which was identified with a natural adenosine by paper chromatography in two solvent systems, mixed melting point, and UV and IR spectrophotometries.

Then, the reaction of 6-thioguanosine (Ib) with hydrazine hydrate was examined. Compound Ib was treated in 60% methylcellosolve solution of hydrazine hydrate and heated at 90—100° for 3 hr. Conversion of Ib to 6-hydrazino derivative was completed and the product (IIb) was separated from reaction mixture in an usual manner in 89% yield. Compound IIb was identified with an authentic 2-amino-6-hydrazino-9-β-D-ribofuranosylpurine[4] by UV and IR spectroscopies. Compound IIb was easily converted to 2,6-diamino-9-β-D-ribofuranosylpurine by 3 hr's refluxing with Raney Ni.

Furthermore, direct replacements of Ia and Ib with several substituted amines and sodium azide were examine as follows. Thus, Ia or Ib was heated with 40% aqueous dimethylamine in seald tube at 100° for 6 hr, only trace of colored material was obtained with large recovery of starting compound. In the cases of ethanolamine, cyclohexylamine and morpholine with Ia, no reaction occurred when heated at 100° for 6—10 hr.

Strong nucleophile, sodium azide was refluxed with Ia in methylcellosolve for 7 hr, the starting material was recovered.

It is worth emphasizing, as a conclusion that this direct hydrazination method of 6-thioderivatives was demonstrated to be conveniently applied to introduction of an amino group to 6-position of purine ribonucleosides *via* mercapto intermediates.

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6) J.A. Johnson Jr, H.J. Thomas, and H.J. Schaffer, *J. Am. Chem. Soc.*, **80**, 699 (1958).

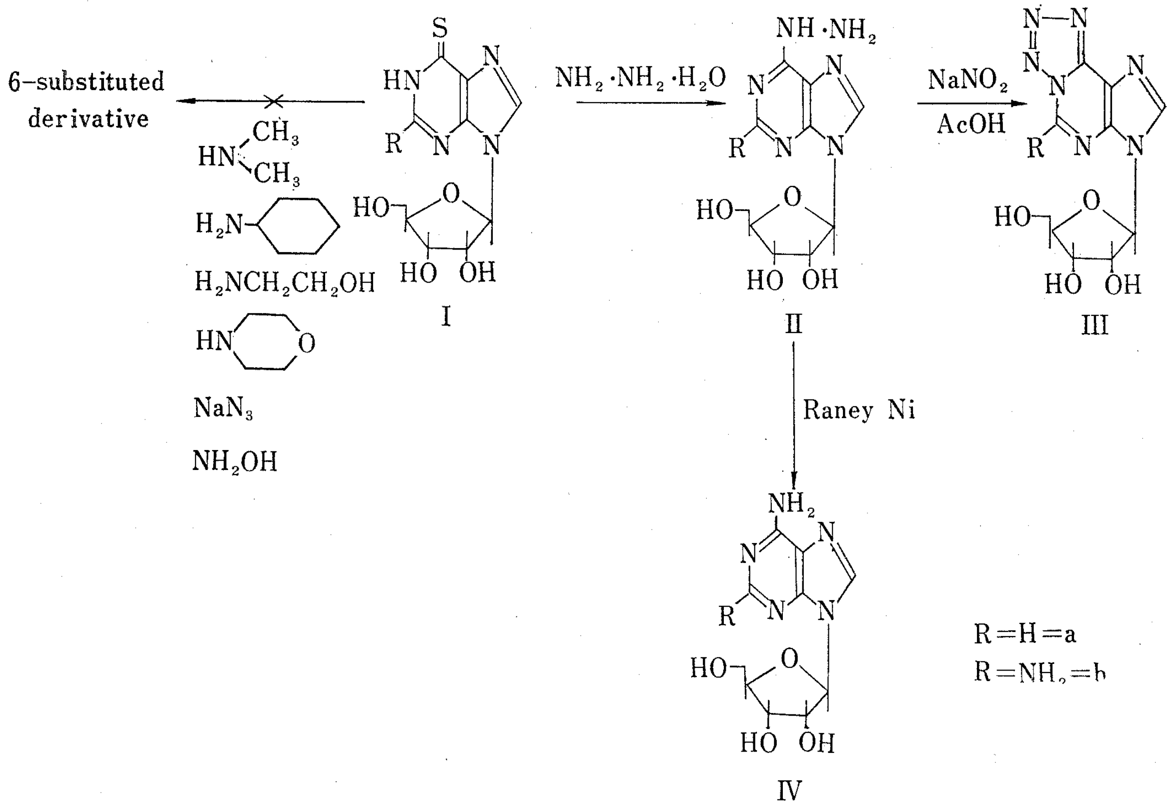

Chart 1

This method is superior to the previous synthetic way, which involves alkylation of thio-derivatives followed by ammonolysis, both in ease in procedure and improvement in chemical yield.

Application of this method in 6-thio-derivative of inosinic and guanylic acid is now under investigation in our laboratory.

Experimental

Paper chromatography were performed on Toyo Roshi No. 51A by ascending method. Solvent systems: A, 95% ethanol–1N sodium acetate (7:3), B, n-butanol–H_2O (86:14). UV spectroscopic measurement was carried out with Carry 14 recording spectrophotometer. All melting points were uncorrected.

TABLE II. Comparison of IIa and IIb with Authentic 6-Hydrazino-9-β-D-ribofuranosylpurine and 2-Amino-6-hydrazino-9-β-D-ribofuranosylpurine

Compound	mp °C	UV, λ_{max} (mμ) ($\varepsilon \times 10^{-3}$)			Rf in Solvent A	B
IIa	214—216	pH 1	261	(15.73)	0.53	0.03
		pH 7	265	(14.80)		
Authentic	214—217	pH 1	261	(15.71)	0.52	0.03
		pH 7	265	(14.75)		
IIb	224—226	pH 7	259	(9.58)	0.38	0.05
			282	(12.50)		
Authentic	224—225	pH 7	259	(9.77)	0.38	0.06
			282	(12.70)		

solvent A: 95% ethanol–1N NaOAc (7:3)
solvent B: n-butanol–H_2O (86:14)

Reaction of 6-Mercapto-9-β-D-ribofuranosylpurine (6-Thioinosine) (Ia) with Hydrazine Hydrate in Methylcellosolve——Compound Ia (2.84 g, 10 mmole) was added to the solution of hydrazine hydrate in methylcellosolve (20 ml) which was contained 13 ml of 95% hydrazine hydrate and 7 ml of methylcellosolve. The resulting clear solution was heated in boiling water–bath for 2 hr. This solution was examined by UV spectrophotometry, the UV absorption maxima at 220 and 317 mμ of the starting solution which was characteristic of Ia was completely disapeared and a new absorption maximum at 265 mμ was observed. Then, the solution was evaporated to dryness under reduced pressure and crystallized from ethanol. White solid which was homogeneous on paper chromatography was obtained. 2.59 g. mp 210—216°. Further recrystallization from absolute ethanol gave white fine needles. Yield 2.22 g. mp 214—216°. *Anal.* Calcd. for $C_{10}H_{14}O_4N_6$: C, 42.54; H, 5.00; N, 29.79. Found: C, 42.81; H, 5.21; N, 29.99. This sample was also identified with authentic 6-hydrazino-9-β-D-ribofuranosylpurine which was proepared by the Johnson's procedure⁶⁾ by mixed melting point test, *Rf* values of paper chromatography in two solvent systems and UV and IR spectrophotometry.

Conversion of IIa to 3-β-D-Ribofuranosyltetrazolo(5,4-i)purine with Nitrous Acid Treatment——To a solution of 0.5 g of IIa in 10 ml of acetic acid, cooled in an ice–bath, was added a solution of 0.14 g of sodium nitrite in 2.5 ml of water under vigorous stirring. White needles began to separate after 10 min; the reaction mixture was kept cold for 1 hr and solid was collected by filtration; Yield, 0.48 g. mp 216—221°. UV λ_{max}^{water} mμ: 251, 259 and 287. This product was identified with authentic tetrazolopurine riboside which was prepared by the method described by Johnson,⁶⁾ *et al.* by UV and IR spectrophotometric measurement. A mixed melting point with an authentic sample showed no depression.

Conversion of IIa to Adenosine by Reduction with Raney Ni——Compound IIa (2.82 g) was dissolved in 100 ml of boiling water and 5 ml of wet Raney Ni was added to this solution. The mixture was refluxed for 3 hr, after this time already conversion was completely, estimated by UV spectroscopic determination. The reaction mixture was filtered and the catalyst was leached several times with 10 ml of boiling water. The filtrate and washings were combined and traeted with active charcoal and refluxed for few minutes. This solution was filtered while hot and concentrated to small volume (*ca.* 25 ml). When the solution was stored in ice–box for 18 hr, white needles began to separate. The crystall was filtered and recrystallized from minimum amount of hot water, white fine needles was obtained. 2.66 g mp 227—229°. This product was identified with natural adenosine by paper chromatography, mixed fusion test, UV and IR spectrophotometries.

Reaction of 6-Chloro-9-β-D-ribofuranosylpurine with Sodium Azide in Methylcellosolve——The suspension of sodium azide (65 mg) in 2.5 ml of methylcellosolve was mixed with 200 mg of 6-chloro-9-β-D-ribofuranosylpurine in 2.0 ml of methylcellosolve. The mixture was heated in boiling water–bath for 0.5 hr and cooled at 4° for 12 hr. The solid was separated and filtered. Recrystallization from hot water gave white micro–needles. Yield, 125 mg mp 220—223°. This product was identified with an authentic tetrazolopurine riboside⁶⁾ by UV and IR spectrophotometry.

Reaction of 2-Amino-6-mercapto-9-β-D-ribofuranosylpurine (6-Thioguanosine) (Ib) with Hydrazine Hydrate in Methylcellosolve——Compound Ib (3 g) was added to the solution of hydrazine hydrate in methylcellosolve (20 ml) which was contained 13 ml of 95% hydrazine hydrate and 7 ml of methylcellosolve. The resulting light–yellow clear solution was heated in boiling water–bath for 3 hr. The reaction mixture was concentrated under reduced pressure to dryness. The resudal gum was treated with 5 ml of 50% ethanol to give white crystalline solid.

The solid was collected and recrystallized from water, white needles was obtained. 2.63 g (89.8%) mp 219—222°. Further crystallization from water gave white needles. mp 224—226°. UV absorption maximum in water; 259 mμ and 282 mμ. This sample was identified with authentic 2-amino-6-hydrazino-9-β-D-ribofuranosylpurine⁴⁾ by UV and IR spectrophotometries.

Conversion of IIb to 2,6-Diamino-9-β-D-ribofuranosylpurine by Reduction with Raney Ni——A mixture of IIb (100 mg) and 0.5 ml of wet Raney Ni in 5 ml of water was refluxed for 3 hr. The reaction mixture was filtered and the catalyst leached several times with 1 ml of boiling water. The filtrate and washings were combined and evaporated under reduced pressure to syrup. This syrup was trituated with ethanol to crystalline mass. Filtration and recrystallization from boiling water to give colorless prism. 87 mg. This sample was identified with authentic 2,6-diamino-9-β-D-ribofuranosylpurine⁴⁾ by paper chromatography, UV and IR spectroscopies.

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