

106. Morio Ikehara, Tohru Ueda, Sumiko Horikawa, and Akihiro Yamazaki :
 Potential Antimetabolites. IV.*¹ Synthesis of 2,6-Bis-alkylthiopurine
 Ribosides and their Selective Substitution by Nucleophilic Reagents.

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The synthesis of mercaptopurine riboside was required both as the interest of potential metabolite antagonist and as a useful intermediate for the synthesis of nucleotide analogs.

Since, it was reported in several metabolite antagonists, such as 6-mercaptopurine and 2-amino-6-mercaptopurine, conversion to the nucleoside increased their activity against tumors.¹⁾ The synthesis of 2,6-bis-alkylthiopurine- β -D-ribofuranoside was first attempted.

Most easily accessible 2-mercaptohypoxanthine²⁾ (I) was thiolated in a yield of 80% by the modified method of Fox, *et al.*¹⁾ 2,6-Bis-mercaptapurine (II) was alkylated with methyl iodide or benzyl chloride in the presence of alkali. Both the bis-alkylthiopurine (IIIa, b) were in good accordance with Montgomery's description.³⁾ In the case of benzylation, the authors obtained, in addition to above (IIIb), a crystalline substance, m.p. 143~144°, which was identified as tribenzyl derivative (IV) from elementary analytical optical behaviors. It has ultraviolet absorption maximum at 230 m μ just like other 9-substituted 2,6-bis-benzylthiopurines.

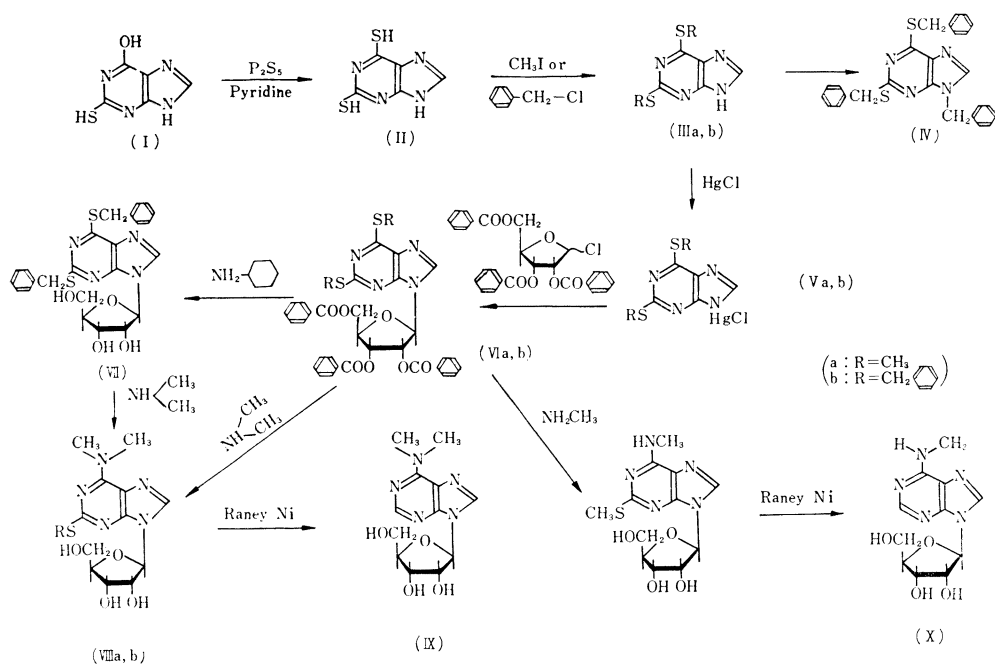


Chart 1.

*¹ Part III. M. Ikehara, N. Nakazawa, H. Nakayama : This Bulletin, **10**, 660(1962).

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1) J. J. Fox, I. Wempen, A. Hampton, I. L. Doerr : J. Am. Chem. Soc., **80**, 1669 (1958).

2) R. K. Robins, *et al.* : *Ibid.*, **75**, 263 (1953).

3) J. A. Montgomery, L. B. Holum, T. P. Johnson : *Ibid.*, **81**, 3963 (1959).

The products (IIIa, b) were converted to the chloromercury salts (Va, b) by the treatment with mercuric chloride in the presence of equimolar amount of sodium hydroxide.

The purification of the ribosidation products of the above mercury salts was achieved by chromatography on alumina. 2,6-Bis-methylthio- (VIa) and 2,6-bis-benzylthio-9-(2',3',5'-tri-O-benzoyl)- β -D-ribofuranosylpurine (VIb) were obtained in a pure state. The former has m.p. 70~80°, $[\alpha]_D^{25}$ -25.0° (yield, 37%) and the latter, m.p. 139~140°, $[\alpha]_D^{25}$ -43.4° (yield, 40%). Both were characterized by elementary analysis, ultraviolet absorption, and further transformation to the known substances. 7-Riboside was not isolated from either of the reaction mixtures, which supports Baker's investigation⁴⁾ concerning the orientation of nucleosidation, in which he stated that if the purine ring has alkylthio group at 2-position, glycosidation occurs exclusively at 9-N position. Upon nucleosidation of (Vb), the steric interference of bulky benzylthio group at 6-position would also be a problem.

Debenzoylation without any change on purine moiety of (VIb) was attempted to obtain nucleoside (VII) by the action of sodium methoxide in methanol, methanol saturated with ammonia, and cyclohexylamine in ethanol. In the last case, resulting crude nucleoside was purified by chromatography on alumina, which afforded a crystalline substance, m.p. 133~135°. Optical behavior of this substance, together with elementary analytical data, proved it to be the desired product, 2,6-bis-benzylthiopurine-9- β -D-ribofuranoside.

These bis-alkylthiopurines were further subjected to direct displacement reaction by a nucleophilic reagent in order to investigate the possibility of utilizing them as the intermediate for 6-substituted purine riboside. As to the substitution at the 6-position of purine nucleoside, Johnson, *et al.*⁵⁾ and Kissman, *et al.*⁶⁾ investigated the reactivity of 6-chloropurine riboside and synthesized various 6-substituted derivatives. Fox's study on thioinosine and thioguanosine suggested the excellent replaceability of thio derivatives. Recently, Robins⁷⁾ converted methylthiopurines to chloro compound to obtain sufficient amount of 6-chloropurines for biological tests. On the replacement of 2,6-bis-alkylthiopurines, Montgomery³⁾ found the remarkable difference of reactivity between 2- and 6-substituents. Expecting simultaneous debenzoylation and 6-substitution, the protected nucleosides (VIa, b) were reacted with dimethylamine at 100°. 2-Methylthio- (VIIIa) and 2-benzylthio-6-dimethylaminopurine riboside (VIIIb) were obtained in 46% and 64% yield, respectively. The physical and photometric constants of (VIIIa) were quite identical with the data of authentic sample.⁸⁾ The structure of (VIIIb) was elucidated from its elementary analysis and optical behavior. Furthermore, the compound (VIIIa) was obtained from the nucleoside (VII) in 84% yield, which proved that the above structure is correct.

Desulfurization of (VIIa) and (VIIIb) was carried out with Raney nickel in boiling ethanol. In both cases, 6-dimethylamino-9- β -D-ribofuranosylpurine (IX) was obtained in ca. 50% yield. The compound (IX) was directly compared with a sample synthesized by authentic procedure.^{9,10)} This fact also confirmed the structure of (VIa, b) as 9- β -D-ribofuranoside and that the replacement had occurred at 6-position.

2,6-Bis-methylthio-9-(2',3',5'-tri-O-benzoyl)- β -D-ribofuranosylpurine (VIa) also affor-

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- 5) J. A. Johnson, H. J. Thomas, H. J. Shaeffer : J. Am. Chem. Soc., **80**, 699 (1958).
- 6) H. M. Kissman, M. J. Weiss : J. Org. Chem., **21**, 1053 (1960).
- 7) R. K. Robins : J. Am. Chem. Soc., **82**, 654 (1960).
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ded 6-methylamino-9- β -D-ribofuranosylpurine (X) by the analogous treatment with methylamine followed by desulfurization with Raney nickel.

From these experiments, 2,6-bis-alkylthio-9-(2',3',5'-tri-O-benzoyl)- β -D-ribofuranosylpurine was proved to be a useful intermediate to the synthesis of coenzyme analogs. Furthermore, the ribosidation reaction of 2,6-bis-alkylthiopurine is one of the shortest route to 9-ribosides.

The biological activity of these substances as cancerostatic and bacteriocidal agents is now under investigation and will be reported elsewhere.

Experimental

2,6-Dimercaptopurine—A solution of 4 g. (1.024 moles) of 2-mercaptopyoxanthine and 16 g. (0.071 mole) of P_2S_5 dissolved in dehyd. pyridine was refluxed for 1.6 hr. After cool, the precipitate thus appeared was separated by decantation and supernatant was evaporated to a syrup. Both the precipitate and syrup were combined and poured into H_2O . After 30 min. of boiling, granular precipitate appeared, which was washed successively with hot H_2O , 50% EtOH, and Et_2O . Dried crude material was obtained in 85% yield and was purified by reprecipitation. When some H_2O was added in original reaction mixture according to the method of Fox,¹⁾ yield dropped to around 50%.

2,6-Bis-benzylthiopurine—To a solution of 4 g. (0.022 mole) of 2,6-dimercaptopurine dissolved in 40 cc. (0.08 mole) of 2*N* NaOH, 6 cc. (0.052 mole) of benzyl chloride was added in small portions with vigorous stirring, and stirring was continued for 2 hr. Resulting precipitate was collected on a filter (5.8 g., 73.4%). UV $\lambda_{max}^{H_2O}$ $m\mu$ (ϵ): 308(12,600), 266(22,700), which were identical with the literature.³⁾ From the mother liquor of above recrystallization a substance, m.p. 142~144°, was obtained. *Anal.* Calcd. for $C_{26}H_{22}N_4S_2$ (tribenzyl derivative): C, 68.71; H, 4.85; N, 12.33. Found: C, 68.36; H, 5.16; N, 12.20. UV λ_{max}^{EtOH} $m\mu$ (ϵ): 308(13,700), 261(25,000), 230(16,400). λ_{min}^{EtOH} $m\mu$ (ϵ): 289(7,400), 247(10,100), 228(15,900).

The dibenzyl derivative could not be obtained when the reaction conditions were altered to 65~70 for 60 min.

Chloromercury Salt of 2,6-Bis-benzylmercaptapurine—To a solution of 2 g. (0.0055 mole) of above bis-benzylthiopurine dissolved in 50 cc. of MeOH, an equimolar 10% NaOH was added. With the addition of 1.5 g. (0.0055 mole) of $HgCl_2$ in 10 cc. of EtOH, a white precipitate formed. This was separated by centrifugation, washed with H_2O and EtOH, and dried.

2,6-Bis-benzylthio-9-(2',3',5'-tri-O-benzoyl)- β -D-ribofuranosylpurine—A suspension of 3.1 g. (0.005 mole) of the above mercury salt in xylene was concentrated to 100 cc. by azeotropical distillation. 2,3,5-Tri-O-benzoyl-D-ribofuranosyl chloride, prepared from 3.0 g. (0.006 mole) of 1-O-actyl sugar,¹¹⁾ in 20 cc. of benzene was added and 20 cc. of the solvent was removed by distillation. After 3 hr. of refluxing, solvent was removed below 40°. Residual red syrup was taken up in 100 cc. of $CHCl_3$, washed with 30% KI and H_2O , and dried over Na_2SO_4 . Evaporation of the solvent gave 4 g. of residue, which was dissolved in benzene and chromatographed over alumina. Elution with benzene-AcOEt gave ca. 700 mg. (16%) of crystals, m.p. 139~140°. When the residue was seeded with the pure product the same crystalline substance was obtained without chromatographical purification. *Anal.* Calcd. for $C_{45}H_{36}O_7N_4S_2$: C, 66.82; H, 4.46; N, 6.93. Found: C, 66.84; H, 4.28; N, 6.52.

UV λ_{max}^{EtOH} μ (ϵ): 310(15,400), 265(36,100), 230(69,000). λ_{min}^{EtOH} μ (ϵ): 292(11,000), 246(21,500). $[\alpha]_D^{15}$: -43.4 ($c=0.465$, dioxane).

2,6-Bis-benzylthio-9- β -D-ribofuranosylpurine—To a suspension of 700 mg. of the above protected nucleoside in 20 cc. of dehyd. MeOH, 2 cc. of cyclohexylamine was added and mixture was allowed to stand for 2 days at room temperature. The reaction was completed by the refluxing and the mixture was evaporated to a syrup, which was co-distilled twice with H_2O to remove cyclohexylamine. The residue was taken up in benzene and triturated with hexane to induce the crystallization of a crude substance (430 mg., yield quantitative). Recrystallization from Me_2CO -hexane gave amorphous powder, m.p. 133~135°. *Anal.* Calcd. for $C_{24}H_{24}O_4N_4S_2$: C, 58.06; H, 4.84; N, 11.29. Found: C, 57.89; H, 4.92; N, 11.11.

$[\alpha]_D^{15}$: -16.1 ($c=0.36$, MeOH). UV λ_{max}^{EtOH} $m\mu$ (ϵ): 311(13,700), 204(25,200), 232(16,800). λ_{min}^{EtOH} $m\mu$ (ϵ): 289(7,850), 245(9,800), 227(15,350).

2-Benzylthio-6-dimethylamino-9- β -D-ribofuranosylpurine—i) A mixture of 1 g. of protected benzoyl nucleoside and 30 cc. of 33% aqueous dimethylamine was sealed in a glass tube, and heated at 100 for 3 hr. The resulting solution was taken out of the tube and evaporated to 1/3 of the

original volume. The separated oil solidified after a while, which was collected by the filtration and recrystallized from EtOH, m.p. 185~186°. (300 mg., 58%). $[\alpha]_D^{20}$: -44.5° (c=0.805, MeOH). *Anal.* Calcd. for C₁₉H₂₃O₄N₃S: C, 54.67; H, 5.51; N, 16.78. Found: C, 54.70; H, 5.41; N, 17.55.

UV $\lambda_{\max}^{\text{EtOH}}$ μ (ϵ): 287(19,300), 249(26,900). $\lambda_{\min}^{\text{EtOH}}$ μ (ϵ): 265(13,750), 228(13,000).

ii) From 2,6-bis-benzyl-9- β -D-ribofuranosylpurine, 2-benzylthio-6-dimethylaminopurine was obtained by analogous manner in 61~87% yield; m.p. 188~189°, when mixed with above sample, m.p. 186~188°.

Chloromercury Salt of 2,6-Bis-methylthiopurine—To 1.0 g. of 2,6-bis-methylthiopurine dissolved in MeOH by warming at 50°, 4.7 cc. of *N* NaOH was added, followed by the addition of 10 cc. of MeOH solution of 1.28 g. of HgCl₂ with stirring. Resulting white precipitate was collected on a filter, washed, and dried at room temperature in a desiccator, m.p. above 200° (decomp.). Yield, 2.0 g. (95%).

2,6-Bis-methylthio-9-(2', 3', 5'-tri-O-benzoyl)- β -D-ribofuranosylpurine—A suspension of 4 g. of above chloromercury salt in 150 cc. of xylene was azeotropically dried by distillation. A solution of 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride, obtained from 5.0 g. of 1-O-acetyl sugar, in 30 cc. of xylene was added and refluxed for 4 hr. with stirring. Xylene was evaporated in a reduced pressure, the residue was extracted with hot CHCl₃, 30% KI solution and H₂O, and dried over Na₂SO₄. Evaporation of CHCl₃ gave 3.0 g. of glassy material, which was purified by alumina chromatography (benzene and AcOEt). Recrystallization from EtOH-H₂O gave a powder, m.p. 70~80° (2.7 g., 37%). *Anal.* Calcd. for C₃₃H₂₈O₇N₄S₂: C, 60.36; H, 4.28; N, 8.53. Found: C, 60.57; H, 4.21; N, 8.06.

UV $\lambda_{\max}^{\text{EtOH}}$ μ (ϵ): 228(57,300), 259(29,900), 308(12,100); $\lambda_{\max}^{0.1N \text{ HCl}}$ μ : 240, 270, 312; $\lambda_{\max}^{0.1N \text{ NaOH}}$ μ : 262, 305. These optical behaviors were quite identical with those taken from each fraction of chromatography, which indicates a negligible occurrence of 7-riboside.

2-Methylthio-6-dimethylamino-9- β -D-ribofuranosylpurine—The above tribenzoyl nucleoside (1.3 g.) was heated in a fused tube with 65 cc. of 35% dimethylamine at 100° for 12 hr. After 3 hr., clear solution was obtained. Small amount of insoluble material was filtered off, and the filtrate was evaporated *in vacuo*. Resulting crystalline mass was recrystallized from EtOH to needles, m.p. 171~172° (310 mg.), UV $\lambda_{\max}^{\text{EtOH}}$ μ : 248, 288; $\lambda_{\max}^{\text{EtOH}}$ μ : 247, 288, which were in good accordance with Baker's description.¹²⁾

6-Dimethylamino-9- β -D-ribofuranosylpurine—i) 2-Benzylthio-6-dimethylamino-9- β -D-ribofuranosylpurine (100 mg.) was refluxed for 2.5 hr. in 5 cc. of EtOH with a small amount of Raney-Ni. The catalyst was filtered, the filtrate and washings were combined, and evaporated *in vacuo*. The residue was taken up in hot Me₂CO and set aside. White fluffy needles, m.p. 180~181°, were obtained (30 mg., 42.5%). This was identified with an authentic specimen,¹²⁾ 182~183°, by mixed melting point test, which showed 180°. Further comparison was made by paper chromatography (solvent, NH₄OH, pH 9), R_f 0.65, authentic sample, 0.65. Ultraviolet absorption of this spot (cut out and eluted with H₂O) was identical with that of an authentic specimen, λ_{\max} : 274 μ ; λ_{\min} : 235 μ .

ii) From 2-methylthio-6-dimethylamino-9- β -D-ribofuranosylpurine: 6-dimethylaminopurine riboside was obtained in 57% yield by an analogous procedure described above. UV: $\lambda_{\max}^{\text{EtOH}}$ 267 μ , $\lambda_{\max}^{\text{EtOH}}$ 274 μ , $\lambda_{\max}^{\text{EtOH}}$ 273 μ .

2-Methylthio-6-methylamino-9- β -D-ribofuranosylpurine—Benzoylated bis-methylthiopurine riboside (1.3 g.) described above was heated with 40 cc. of methylamine in a fused tube at 100° for 4.5 hr. Brown-red solution was filtered and the filtrate was evaporated *in vacuo*. A colored syrup thus obtained was triturated with H₂O and extracted with CHCl₃ (3×30 cc.). H₂O-layer was evaporated *in vacuo* to yield 300 mg. of a substance. UV: $\lambda_{\max}^{0.1N \text{ HCl}}$ 271 μ ; $\lambda_{\max}^{\text{EtOH}}$ 279, 240; $\lambda_{\max}^{0.1N \text{ NaOH}}$ 279, 239.

Picrate: Recrystallized from MeOH, m.p. 158~160°, by rapid heating. It decomposed at 223° by a gentle heating.

6-Methylamino-9- β -D-ribofuranosylpurine—To a solution of 200 mg. of above 2-methylthio-6-methylaminopurine riboside in 30 cc. of EtOH, 3 g. of Raney-Ni was added and refluxed for 5 hr. with stirring. Ni was removed by filtration, the filtrate and washings (hot EtOH) were combined, and evaporated *in vacuo* to yield 50 mg. of product. UV $\lambda_{\max}^{0.1N \text{ HCl}}$ 262 μ ; $\lambda_{\max}^{\text{EtOH}}$ 265 μ ; $\lambda_{\max}^{0.1N \text{ NaOH}}$ 265 μ . The R_f value of paper chromatography with PrOH-NH₃-H₂O (60:30:10), 0.36. These data are in good accordance with those of an authentic sample.¹³⁾

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Summary

2,6-Bis-methylthio- and 2,6-bis-benzylthio-9-(2', 3', 5'-tri-O-benzoyl)- β -D-ribofuranosylpurines were synthesized by the condensation of chloromercury salt of 2,6-bis-alkylthiopurines with 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride. The transformation to 6-dimethylamino- and 6-methylamino-9- β -D-ribofuranosylpurine by the successive amination and desulfurization was achieved.

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107. Shozo Kamiya : Azidoquinoline and Azidopyridine Derivatives. IV.*¹
Reactions of Azido Group in the Quaternary Salts of
4-Azidoquinoline, 4-Azidopyridine, and their 1-Oxides.

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In general, quaternary salts of pyridine and quinoline derivatives have increased polar effect in their nitrogen and the reactivity of 2- and 4-positions to nucleophilic reagents is further potentiated. This reactivity is further increased in the quaternary salts of their N-oxide derivatives.

Ochiai and his co-workers¹⁾ obtained 4-substituted pyridines and quinolines by alkaline decomposition of the methiodide of 4-substituted pyridine and quinoline 1-oxides. Okamoto,²⁾ Tani,³⁾ and Feely⁴⁾ obtained numerous cyanopyridine derivatives by the reaction of potassium cyanide with the quaternary salt of various pyridine 1-oxide derivatives, thereby developing a new field in the chemistry of quaternary salts.

Previously, the present author synthesized 4-azidoquinoline 1-oxide and 4-azidopyridine 1-oxide, and reaction of the azido group in these compounds was examined.⁵⁾ In the present paper, reaction of the quaternary salts of 4-azido-quinoline and -pyridine, and their 1-oxides will be described.

4-Azidoquinoline (I) reacts with methyl iodide or dimethyl sulfate in chloroform, at room temperature, and quantitatively forms the quaternary salts (IIa and IIb).

On the other hand, if 4-azidoquinoline 1-oxide (III) is refluxed with methyl iodide in chloroform, only a small amount of 4-azidoquinoline and 4,4'-azidoquinoline are formed and the majority of the starting material is recovered unchanged. Reaction of (III) with dimethyl sulfate in chloroform, at room temperature, affords 1-methoxy-4-azidoquinolinium methosulfate (IVa). The use of diethyl sulfate gives 1-ethoxy-4-azidoquinolinium methosulfate (IVb).

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