[Chem. Pharm. Bull. 22(2) 342—348 (1974)]

UDC 547.963.32.057 : 547.857.04

Synthesis of Tricyclic Ribonucleosides

Ryuji Marumoto, Yoshio Yoshioka, and Mikio Honjo

Chemical Research Laboratories, Central Research Division, Takeda Chemical Industries, Ltd.[1]

(Received May 14, 1973)

Tricyclic ribonucleosides which possess novel heterocyclic systems such as thiazolo[3,2-*a*]purine (XI), dihydrothiazolo[3,2-*a*]purine (VI), dihydro-*m*-thiazino[3,2-*a*]purine and *s*-triazolo[4,3-*a*]purine were synthesized starting from 2-mercaptoinosine. These tricyclic ribonucleosides were somewhat less stable than guanosine toward the acid hydrolysis of nucleosidic linkages.

Since discoveries of unusual fluorescent nucleosides in *t*-RNA's[2] and structure elucidations of their bases Y and Yt,[3] much attention has been directed toward the synthesis of tricyclic ribonucleosides[4] which possess the third heterocycle fused to the purine moiety at N^1- and C_2-positions.[5] This paper deals with the synthesis of this kind of ribonucleosides in connection with our earlier work on the synthesis of 2-mercaptoinosine derivatives.[6]

Reaction of an aqueous solution of 2-mercaptoinosine potassium salt (I) with an excess of α,ω-dibromoalkanes (II), $Br(CH_2)_nBr$ ($n=2$—4), in the presence of alkali at room temperature afforded UV absorbing compounds which were detectable by paper electrophoresis (borate buffer, pH 9.2). In the case $n=2$ (or 3), the major product was isolated in an excellent yield as colorless needles and the structure was established to be 3-β-D-ribofuranosyl-6,7-dihydrothiazolo[3,2-*a*]purine-9-one (III) (or 3-β-D-ribofuranosyl-6,7-dihydro-*m*-thiazino[3,2-*a*]purine-10-one (IV)) on the basis of its elemental analysis, nuclear magnetic resonance (NMR) and ultraviolet (UV) spectra. The minor product, which had a faster mobility on paper electrophoreogram, was assumed to be α,ω-bis-(2-thioinosinyl)alkane on the basis of its mobility and UV spectra.[7] In the case $n=4$, however, the major product was bis-nucleosidyl alkane and the minor was the corresponding tricyclic nucleoside. When $n=5$, the reaction afforded 1,5-bis(2-thioinosinyl)pentane (V) as a sole product. These data show that the formation of tricyclic nucleosides decreases as the chain length of α,ω-dihalogenoalkanes increases. The reaction between I and II in the absence of alkali, however, resulted in a predominant formation of bisnucleosidyl alkanes even in the reaction with ethylene dibromide. The structure

---

[1] Location: *Juso-Nishinocho, Higashiyodogawa-ku, Osaka.*

[2] *a*) U.L. RajBhandary, S.H. Chang, A. Stuart, R.D. Faulkner, R.M. Hoskinson, and H.G. Khorana, *Proc. Natl. Acad. Sci.*, **57**, 751 (1967); *b*) B.S. Dudock, G. Katz, E.K. Taylor, and R.W. Holley, *ibid.*, **62**, 941 (1969); *c*) L.M. Fink, T. Goto, F. Frankel, and I.B. Weinstein, *Biochem. Biophys. Res. Commun.*, **32**, 963 (1968).

[3] *a*) K. Nakanishi, N. Furutachi, M. Funamizu, D. Grunberger, and I.B. Weinstein, *J. Am. Chem. Soc.*, **92**, 7617 (1970); *b*) H. Kasai, M. Goto, S. Takemura, T. Goto, and S. Matsuura, *Tetrahedron Letters*, **1971**, 2725.

[4] *a*) R. Shapiro and J. Hachmann, *Biochem.*, **5**, 2799 (1966); *b*) M.J. Robins, R.H. Hall, and R. Thedford, *ibid.*, **6**, 1837 (1967); *c*) J.A. Johnson, Jr., H.J. Thomas, and H.J. Schaefer, *J. Am. Chem. Soc.*, **80**, 699 (1958); *d*) R.L. Tolman and L.B. Townsend, *Tetrahedron Letters*, **1968**, 4815; *e*) K.H. Schram and L.B. Townsend, *Tetrahedron Letters*, **1971**, 4757; *f*) J.R. Barrio, J.A. Secrist, and N.J. Leonard, *Biochem. Biophys. Res. Commun.*, **46**, 597 (1972).

[5] *a*) B.M. Goldschmidt, T.P. Blazej, and B.L. Van Duuren, *Tetrahedron Letters*, **1968**, 1583; *b*) E. Zbiral and E. Hugel, *ibid.*, **1972**, 439 (1972).

[6] *a*) A. Yamazaki, I. Kumashiro, and T. Takenishi, *J. Org. Chem.*, 32, 3032 (1967); *b*) K. Imai, R. Marumoto, K. Kobayashi, Y. Yoshioka, J. Toda, and M. Honjo, *Chem. Pharm. Bull.* (Tokyo), **19**, 576 (1971).

[7] α,ω-Bis(2-thioinosinyl)alkanes ($n=1$ and 3) have already been prepared in our laboratory (ref. 6*b*)).

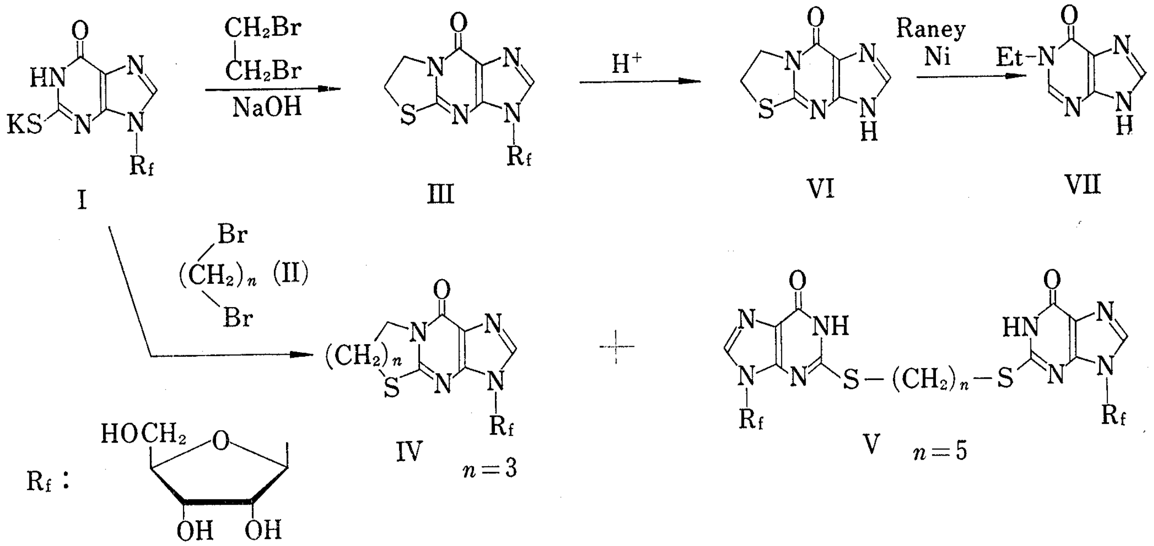

Chart 1

III receives support from the experiment that III was refluxed in alcoholic hydrochloric acid to yield 6,7-dihydrothiazolo[3,2-*a*]purine-9-one (VI), which was converted to N^1-ethylhypoxanthine[8] (VII) by desulfurization with Raney nickel (Chart 1).

Attempts to synthesize 3-*β*-D-ribofuranosyl-thiazolo[3,2-*a*]purine-9-one (VIII) by a similar reaction of I with dibromoethylene (a mixture of *cis*- and *trans*-isomers) in water or methanol in the presence of alkali were unsuccessful and I was recovered. However, the reaction of I with dibromoethylene in dimethylformamide in the presence of sodium ethoxide afforded two UV absorbing compounds, which were purified by column chromatography on activated charcoal. One of them possessing a faster mobility on paper electrophoresis was assigned 2-(*cis*-*β*-bromovinylthio)inosine (IX) on the basis of the elemental analysis, the UV spectra very similar to those of 2-alkylthioinosine, and the NMR spectrum (*cis* vinyl protons, $J_{ab}=6.5$ Hz). The structure VIII was assigned to the other compound on the basis of the elemental analysis, UV spectra (a fine structure near 300 nm characteristic of tricyclic anthracene) and NMR spectrum (*cis* vinyl protons of thiazole ring, $J_{ab}=5$ Hz). The compound (IX) was proved to be an intermediate for the production of VIII, because heating of the former in methylcellosolve in the presence of sodium acetate led to formation of the latter quantitatively. A similar reaction of I with *cis*-dichloroethylene afforded both VIII and the chloro analog (X) of IX. However, the reaction of I with *trans*-dichloroethylene led only to recovery of I. This fact indicates a facile formation of halogenoacetylene from *cis*-dihalogenoethylene (not from *trans*-isomer)[9] followed by addition of halogenoacetylene to I to yield IX or X. Treatment of VIII with alcoholic hydrochloric acid afforded thiazolo-[3,2-*a*]purine-9-one (XI), which was converted by thiation with phosphorus pentasulfide to thiazolo[3,2-*a*]purine-9-thione (XII), a novel tricyclic compound related to 6-mercaptopurine.

An alternative ring closure reaction of IX to yield thiazolo[2,3-*b*]purine (XVIII) was excluded, probably because of a steric hindrance between the thiazolo ring and the ribose moiety. A similar steric hindrance can be seen in methylation of guanosine, which gives N^1- and N^7-methylguanosine and no N^3-methyl derivative.[10] Attempts to prove unambiguity of the structure VIII were unsuccessful: 1) hydrogenation of VIII with Raney nickel did not yield N^1-vinylinosine, but inosine. 2) Acetonization of VIII to the 2′,3′-O-isopropylidene derivative (XIX), followed by tosylation gave the 2′,3′-O-isopropylidene-5′-

---

[8] R.W. Balsiger, A.L. Fikes, T.P. Johnston, and J.A. Montgomery, *J. Org. Chem.*, **26**, 3446 (1961).
[9] J. Flynn, V.V. Badiger, and W.E. Truce, *J. Org. Chem.*, **28**, 2298 (1963).
[10] A.D. Broom, L.B. Townsend, J.W. Jones, and R.K. Robins, *Biochemistry*, **3**, 494 (1964).

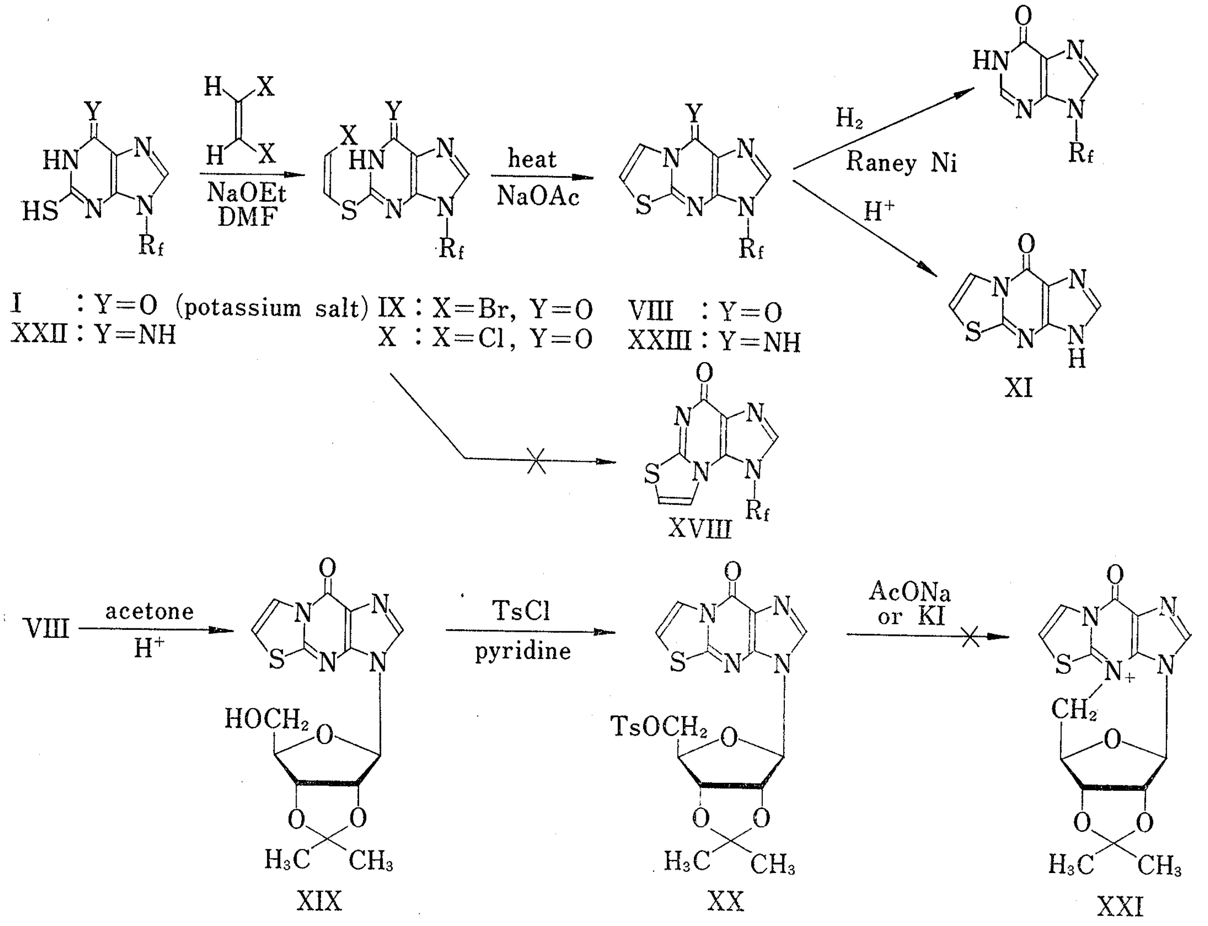

Chart 2

O-tosyl derivative (XX). Treatment of XX with sodium acetate or potassium iodide[11] did not lead to the quarternary salt (XXI) (Chart 2).

Reaction of XI with bromine in an aqueous solution of disodium monohydrogen phosphate furnished a monobromo derivative (XIII). The presence of bromine on the thiazole ring was evidenced by disappearance of an AB-type absorption at δ 7.4 and 8.0 ppm, $J_{ab}=5$ Hz in the NMR spectrum (d_6-DMSO). Reaction of XI with hydrazine hydrate afforded colorless needles, mp>300°. The structure N^1-aminoguanine was assigned to this compound (XIV), on the basis of the elemental analysis ($C_5H_6ON_6 \cdot {}^1/_2H_2O$), the NMR spectrum ($d_6$-DMSO) showing the presence of two amino groups at δ 5.4 and 6.9 ppm and one aromatic proton at δ 7.8 ppm, and the mass ($M^+=166$), and UV spectra. The formation of XIV would be explained by a mechanism as shown in Chart 3: Nucleophilic attack of hydrazine on XI causes cleavage of the thiazole ring to give N^1,2-disubstituted hypoxanthine (XV). Further attack of hydrazine on XV results in ring-opening of the pyrimidine ring to yield 4,5-disubstituted imidazole (XVI). Ring closure of XVI to N^1-amino-2-hydrazinohypoxanthine (XVII), followed by reduction with hydrazine leads to XIV (Chart 3).

Reaction of 2-mercaptoadenosine[12] (XXII) with *cis*-dichloroethylene afforded a tricyclic adenosine derivative, 9-imino-3-β-D-ribofuranosylthiazolo[3,2-*a*]purine (XXIII).

Synthesis of a tricyclic inosine derivative possessing *s*-triazole ring involves the reaction of 2-hydrazinoinosine (XXV) with ethyl orthoformate. XXV was derived from 2-bromoinosine (XXIV) which was obtained by treatment of I with bromine in hydrobromic acid.

---

[11] V.M. Clark, A.R. Todd, and J. Zussman, *J. Chem. Soc.*, **1951**, 2952.
[12] Y. Yoshioka, unpublished.

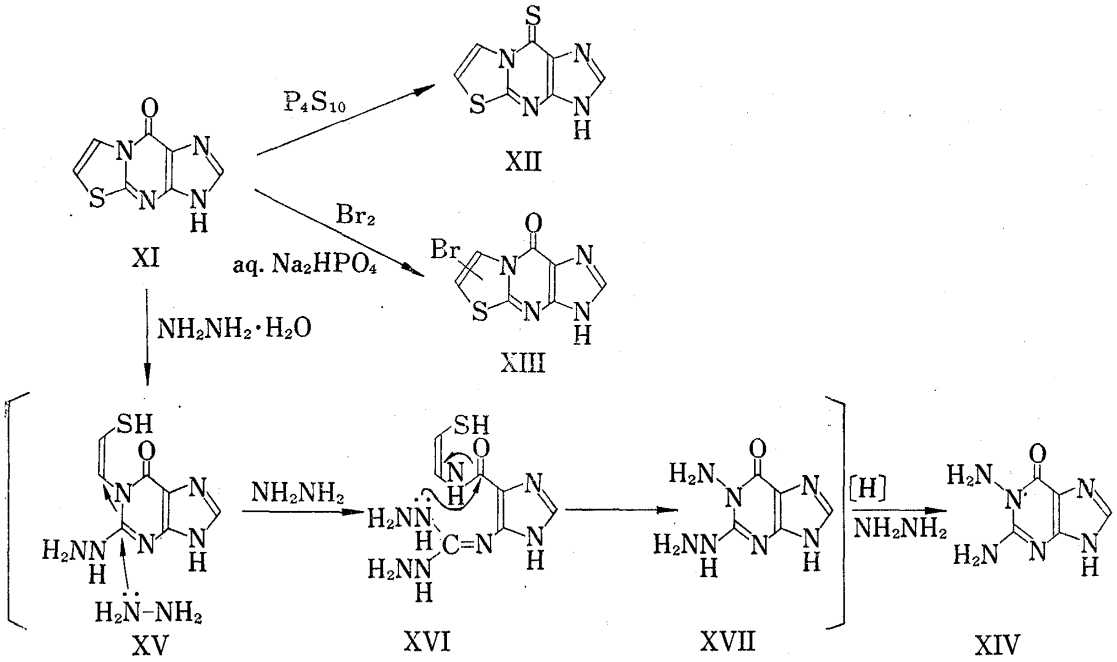

Chart 3

Reaction of XXV with ethyl orthoformate in dimethylformamide afforded two compounds, which were purified by DEAE-cellulose column chromatography and had the same UV spectrum characteristic of tricyclic compounds near 300 nm. They were assigned the structures, 3-*β*-D-ribofuranosyl-*s*-triazolo[4,3-*a*]purine-9-one (XXVI) and the 2′,3′-O-ethoxymethylidene derivative (XXVII), respectively, on the basis of the elemental analyses, NMR spectra and color reactions with periodate-benzidine[13] (Chart 4).

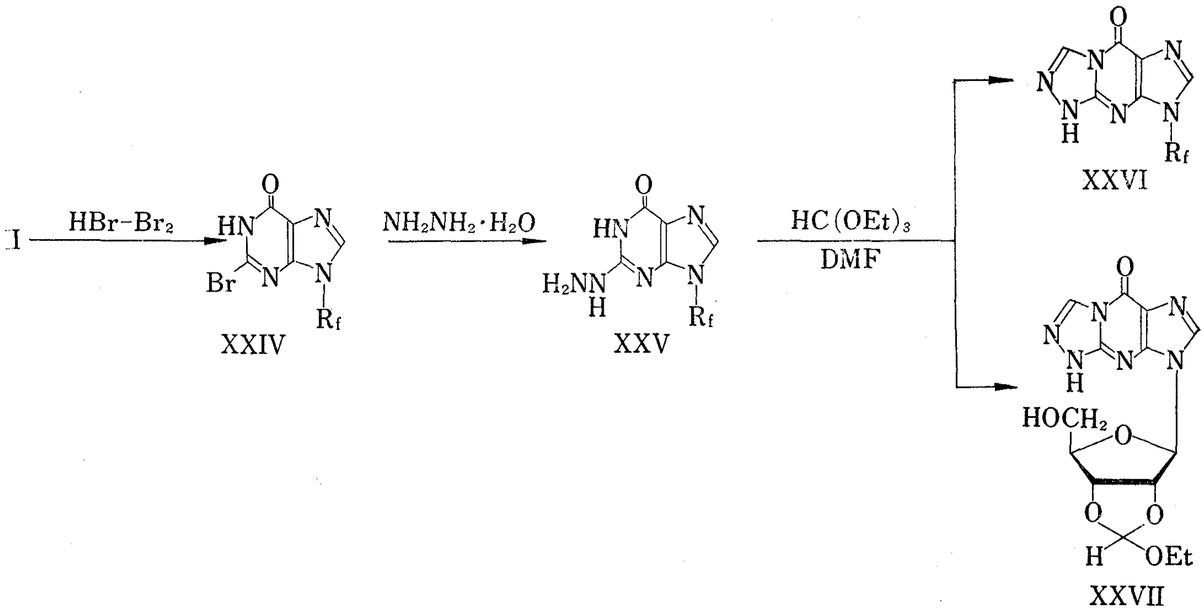

Chart 4

These tricyclic ribonucleosides (VIII, XXIII and XXVI) were strongly fluorescent under ultraviolet light, and all XXVI, VIII, III and IV were somewhat less stable than guanosine against acid hydrolysis with regard to their glycosidic linkages.

---

[13] M. Viscontini, D. Hoch, and P. Karrer, *Helv. Chim. Acta*, **38**, 642 (1955).

Experimental[14]

3-β-D-Ribofuranosyl-6,7-dihydrothiazolo[3,2-*a*]purine-9-one (III)——Compound (I)[6] (680 mg, 2 mmoles) was dissolved in water (10 ml), to which were added 1 N NaOH (4 ml) and 1,2-dibromoethane (0.4 ml, 4 m-moles). The mixture was stirred at room temperature for 16 hr, and neutralized with IR-120 (H^+ form). The resin was removed by filtration and washed thoroughly with water. The filtrate and washings were combined and applied to a column of Dowex 1×8 ($B_4O_7^{--}$ form, 200 ml). The column was washed with water, then eluted with a mixture of 0.1 M borax and 0.1 M NaCl (1:1 v/v). The eluate (TOD_{260}[15] 17800) was adjusted to pH 3, and adsorbed on a column of activated charcoal (2 g). The column was washed with water and eluted with a mixture of EtOH, H_2O, *n*-BuOH and 28% NH_4OH (45:48:5:2 v/v). The eluate was evaporated to dryness *in vacuo* and the residue was added with EtOH. Crude crystalls were recrystallized from water to give colorless fine needles (200 mg), mp 220—221°. $[\alpha]_D^{22}$ −29.0° (c=1.0, DMF). UV $\lambda_{\max}^{\mathrm{pH2}}$ nm (ε): 265 (1.75×10^4), 280 (sh); $\lambda_{\max}^{\mathrm{pH12}}$ nm (ε): 264.5 (1.75×10^4), 280 (sh); $\lambda_{\max}^{\mathrm{pH2\text{-}12}}$ nm: 237. NMR (d_6-DMSO) δ: 5.80 (1H, d, J=5 Hz, H_1'); 8.20 (1H, s, H_2). *Anal.* Calcd. for $C_{12}H_{14}O_5N_4S$: C, 44.17; H, 4.29; N, 17.18. Found: C, 44.15; H, 4.15; N, 16.92.

3-β-D-Ribofuranosyl-6,7-dihydro-*m*-thiazino[3,2-*a*]purine-10-one (IV)——A mixture of I (680 mg, 2 mmoles), 1 N NaOH (8 ml) and 1,3-dibromopropane (0.4 ml) was treated in the similar manner as described in the section of III. Colorless fine needles (100 mg), mp 206—207° were obtained. UV $\lambda_{\max}^{\mathrm{pH1}}$ nm (ε): 283 (1.43×10^4); $\lambda_{\max}^{\mathrm{pH12}}$ nm (ε): 267 (1.21×10^4), 286 (1.17×10^4). *Anal.* Calcd. for $C_{13}H_{16}O_5N_4S$: C, 45.87; H, 4.74; N, 16.46. Found: C, 46.04; H, 4.72; N, 16.74. The reaction mixture, just before the adsorption on Dowex 1 column, showed the presence of two UV absorbing compounds on paper electrophoreogram. The faster travelling compound (10% yield) was identified as 1,3-bis(2-thioinosinyl)propane by comparing its mobility and UV spectra with those of the authentic sample.[6] The slower travelling compound was found to be IV.

1,5-Bis(2-thioinosinyl)pentane (V)——A mixture of I (680 mg, 2 mmoles), 1,5-dibromopentane (0.4 ml) and 1 N NaOH (8 ml) was stirred at room temperature for 20 hr, neutralized with 1 N H_2SO_4 and evaporated to dryness *in vacuo*. The residue was triturated with water to give crystallines (400 mg), mp 211°. UV $\lambda_{\max}^{\mathrm{pH7}}$ nm: 264, 280 (sh); $\lambda_{\max}^{\mathrm{pH12}}$ nm: 227, 272. *Anal.* Calcd. for $C_{25}H_{32}O_{10}N_8S_2\cdot2H_2O$: C, 42.60; H, 5.15; N, 15.90. Found: C, 41.93; H, 4.77; N, 15.22.

6,7-Dihydrothiazolo[3,2-*a*]purine-9-one (VI)·Hydrochloride——A suspension of III (1.8 g) in 10% ethanolic HCl (50 ml) was refluxed for 2 hr. The crystalls were collected by filtration and washed with EtOH to obtain colorless needles (1.1 g), mp 300—310° (decomp.). UV $\lambda_{\max}^{\mathrm{pH1}}$ nm (ε): 226 (9.43×10^3), 264 (1.50×10^4); $\lambda_{\max}^{\mathrm{pH12}}$ nm (ε): 226 (2.04×10^4), 271 (1.24×10^4). *Anal.* Calcd. for $C_7H_6ON_4S\cdot HCl$: C, 36.45; H, 3.03; N, 24.29; S, 13.87. Found: C, 36.33; H, 2.88; N, 24.01; S, 14.07.

N^1-Ethylhypoxanthine (VII)——A suspension of III (500 mg) in *n*-BuOH (30 ml), after the addition of Raney nickel (3 g), was refluxed for 20 hr. Raney nickel was removed by filtration and the filtrate, after the addition of freshly prepared Raney nickel (2.5 g), was refluxed for 20 hr. Raney nickel was filtered and washed with hot *n*-BuOH. Concentration of the combined filtrate and washings afforded a white solid, which was recrystallized from EtOH to give crystallines (90 mg), mp 276—278°. UV $\lambda_{\max}$ nm: 251 (pH 1), 250 (pH 5), 261 (pH 12). *Anal.* Calcd. for $C_7H_8ON_4$: C, 51.20; H, 4.91; N, 34.13. Found: C, 51.13; H, 4.74; N, 33.79.

2-(*cis*-β-Bromovinylthio)inosine (IX)——To a solution of I (8 g) in DMF (100 ml) was added 1,2-dibromoethylene (a mixture of *cis*(62%)- and *trans*(38%)-isomers) and 1 N NaOEt (40 ml). The mixture was stirred at room temperature for 16 hr and poured into ice water (100 ml). The solution was adjusted to pH 3, and applied to a column of activated charcoal (80 g). The column was washed with water and eluted with a mixture of EtOH, H_2O, *n*-BuOH and 28% NH_4OH (45:48:5:2 v/v). The eluate was evaporated to dryness. The residue was recrystallized from water to give colorless needles (2 g), mp 174—176° (decomp.). $[\alpha]_D^{22}$ +14.8° (c=0.5, DMF). UV $\lambda_{\max}^{\mathrm{pH1}}$ nm (ε): 237 (9.72×10^3), 283 (1.66×10^4); $\lambda_{\max}^{\mathrm{pH13}}$ nm (ε): 238 (1.98×10^4), 278 (1.78×10^4). NMR (d_6-DMSO) δ: 5.89 (1H, d, J=5 Hz, H_1'), 6.95 (1H, d, J=6.5 Hz, vinyl H), 7.93 (1H, d, J=6.5 Hz, vinyl H), 8.28 (1H, s, H_8). *Anal.* Calcd. for $C_{12}H_{13}O_5N_4SBr$: C, 35.56; H, 3.23; N, 13.83; S, 7.91; Br, 19.72. Found: C, 34.88; H, 3.08; N, 13.41; S, 7.73; Br, 20.01.

2-(*cis*-β-Chlorovinylthio)inosine (X)——A mixture of I (0.5 g), *cis*-dichloroethylene (0.5 ml), 1 N NaOEt (2.5 ml) and DMF (5 ml) was treated in the similar manner as described in the section of IX to afford crystallines (0.2 g). mp 188—190°. *Anal.* Calcd. for $C_{12}H_{13}O_5N_4SCl\cdot1/2H_2O$: C, 38.97; H, 3.81; N, 15.15; S, 8.67; Cl, 9.58. Found: C, 38.75; H, 3.42; N, 15.14; S, 8.82; Cl, 9.04.

3-β-D-Ribofuranosylthiazolo[3,2-*a*]purine-9-one (VIII)——i) The column of activated charcoal in the section of IX was secondly eluted with a mixture of *n*-BuOH saturated with water, and pyridine (1:1 v/v)

---

14) All melting points were uncorrected. Paper electrophoresis (PE) was carried out on Whatman No. 1 filter paper at 22 v/cm for 1 hr using 0.05 M borate buffer (pH 9.2).

15) Optical Density (light path length, 1 cm) at 260 nm × ml.

(1 liter). The eluate was evaporated to dryness and the residue was recrystallized from water to give colorless needles (2.3 g), mp 215—216°. $[\alpha]_{\text{D}}^{22}$ +66° (c=1.0, DMF). UV $\lambda_{\max}^{\text{PH1}}$ nm (ε): 230 (2.32×10^4), 290 (sh), 299 (1.37×10^4), 310 (1.19×10^4); $\lambda_{\max}^{\text{PH13}}$ nm (ε): 231 (2.62×10^4), 280 (1.24×10^4), 303 (1.18×10^4), 310 (sh). NMR (d_6-DMSO) δ: 5.94 (1H, d, J=5 Hz, $H_1{}'$), 7.45 (1H, d, J=5 Hz, thiazol ring H), 8.10 (1H, d, J=5 Hz, thiazol ring H), 8.38 (1H, s, H_2). *Anal.* Calcd. for $C_{12}H_{12}O_5N_4S$: C, 44.44; H, 3.73; N, 17.28. Found: C, 44.38; H, 3.53; N, 17.25. ii) A solution of IX (3 g) and NaOAc·$3H_2O$ (3 g) in methyl cellosolve (100 ml) was heated at 120° for 6 hr. The reaction mixture was evaporated to dryness to give colorless needles (2.5 g), mp 215—216°. iii) The column of activated charcoal described in the section of X was secondly eluted with a mixture of n-BuOH saturated with H_2O, and pyridine (1: 1 v/v). The eluate was treated in the similar manner as described in i) to yield VIII (0.2 g).

Desulfurization of VIII (Formation of Inosine)——A suspension of VIII (200 mg) and Raney nickel (2 g) in n-BuOH (20 ml) was refluxed for 20 hr. Raney nickel was filtered off. The filtrate showed on PE the presence of another compound (50% yield, M_{IIIV}=1.8). The product was identified as inosine by comparing its mobility on PE, UV spectra ($\lambda_{\max}$ nm: 249 (pH 5), 252 (pH 12)) and color reaction with periodate-benzidine[12] with those of the authentic sample.

Thiazolo[3,2-*a*]purine-9-one (XI)·Hydrochloride——A suspension of VIII (1 g) in 10% methanolic HCl was refluxed for 1 hr. The pale yellow crystallines were collected by filtration and washed with EtOH (710 mg), mp>300°. UV $\lambda_{\max}^{\text{PH1}}$ nm (ε): 229 (2.09×10^4), 284 (1.09×10^4), 297 (1.25×10^4), 310 (1.12×10^4); $\lambda_{\max}^{\text{PH12}}$ nm (ε): 243 (3.44×10^4), 290 (9.48×10^3), 3.03 (9.19×10^3). *Anal.* Calcd. for $C_7H_4ON_4S\cdot HCl$: C, 36.76; H, 2.20; N, 24.50. Found: C, 36.94; H, 2.26; N, 23.86.

Thiazolo[3,2-*a*]purine-9-thione (XII)——A mixture of XI (500 mg) and P_4S_{10} (1 g) in pyridine (10 ml) was refluxed for 1 hr, and pyridine was distilled off *in vacuo*. The residue was added with water (100 ml) and the mixture was refluxed for 30 min. After cooling the precipitate was collected by filtration, washed with MeOH, and dissolved in 0.1 N NaOH (50 ml). The insoluble material was filtered off and the filtrate was neutralized to give pale brown solid, which was recrystallized from water to yield yellow needles (80 mg), mp>300°. UV $\lambda_{\max}^{\text{PH1}}$ nm (ε): 230 (1.93×10^4), 273 (1.41×10^4), 357 (1.60×10^4), 370 (1.83×10^4); $\lambda_{\max}^{\text{PH12}}$ nm (ε): 239 (1.44×10^4), 277 (2.01×10^4), 347 (1.55×10^4), 360 (1.72×10^4). *Anal.* Calcd. for $C_7H_4N_4S_2$: C, 40.38; H, 1.92; N, 26.92; S, 30.77. Found: C, 40.03; H, 1.97; N, 26.32; S, 30.82.

Monobromoderivative (XIII) of XI——Bromine (1 ml) was added dropwise to a stirred suspension of XI (0.5 g) in a mixture of 0.2 M Na_2HPO_4 and dioxane (1: 1 v/v, 100 ml). The reaction mixture was kept at room temperature for 20 hr and evaporated to dryness. The residue was triturated with water to give white solid. This solid was dissolved in EtOH and the solution was added with small amount of water. A white insoluble material was filtered off and the filtrate was added with a small amount of water and kept in the refrigerator to afford colorless needles (150 mg), mp 220—230° (decomp.). UV $\lambda_{\max}^{\text{MeOH}}$ nm: 226, 260, 302. NMR (d_6-DMSO) δ: 6.70 (1H, s), 7.42 (1H, s). *Anal.* Calcd. for $C_7H_3ON_4SBr$: N, 20.67; Br, 29.48. Found: N, 20.38; Br, 32.85.

1-Aminoguanine (XIV)——A mixture of XI (0.2 g) and 10% aq. NH_2NH_2 (10 ml) was heated at 130° for 8 hr. After cooling, the colorless needles were collected and washed with water. Yield was 50 mg, mp >300°. UV $\lambda_{\max}^{\text{PH1}}$ nm: 249, 276; $\lambda_{\max}^{\text{PH13}}$ nm: 245, 278; $\lambda_{\min}^{\text{PH1}}$ nm: 266; $\lambda_{\min}^{\text{PH12}}$ nm: 262. NMR (d_6-DMSO) δ: 5.35 (2H, broad s, 1–NH_2), 6.83 (2H, broad s, 2–NH_2), 7.70 (1H, s, H_8). Mass Spectrum m/e: 166 (M^+), 135. *Anal.* Calcd. for $C_5H_6ON_6\cdot 1/2H_2O$: C, 34.28; H, 3.99; N, 47.98. Found: C, 34.56; H, 3.66; N, 46.86.

2′,3′-O-Isopropylidene-3-*β*-D-ribofuranosylthiazolo[3,2-*a*]purine-9-one (XIX)——Pyrophosphoryl chloride (1 ml) was added at 0—5° dropwise to an ice-cold stirred suspension of VIII (2.3 g) in acetone (100 ml). The mixture was stirred at this temperature for 2 hr. The clear solution was poured into cold 3% NH_4OH (25 ml), evaporated *in vacuo* to distill off ammonia and adjusted to pH 3. The solution was desalted with a column of activated charcoal (20 g) (effluent: n-BuOH saturated with H_2O 190 ml+28% NH_4OH 10 ml+pyridine 50 ml). The eluate was evaporated to dryness to afford hygroscopic powder (2.5 g).

2′,3′-O-Isopropylidene-5′-O-tosyl-3-*β*-D-ribofuranosylthiazolo[3,2-*a*]purine-9-one (XX)——Tosyl chloride (1.2 g) was added dropwise to a stirred solution of XIX (1 g) in pyridine (20 ml). The reaction mixture was kept at room temperature for 20 hr, poured into aq. saturated $NaHCO_3$ (200 ml), and extracted with $CHCl_3$ (200 ml × 2). The $CHCl_3$ layer was washed with water, dried with anhydrous Na_2SO_4, and purified by silica gel column chromatography (silica gel 30 g, $CHCl_3$–MeOH 11: 1 v/v). The fractions were collected and evaporated to dryness to give pale pink powder (360 mg). NMR ($CDCl_3$) δ: 1.37 and 1.59 (6H, 2s, isopropyl idene group), 2.36 (3H, s, tosyl), 5.02 (1H, double d, $J_{2',3'}$=6 Hz, $J_{3',4'}$=3 Hz, $H_{3'}$), 5.29 (1H, double d, $J_{1',2'}$=2.5 Hz, $J_{2',3'}$=6 Hz, $H_{2'}$), 6.06 (1H, d, J=2.5 Hz, $H_{1'}$). *Anal.* Calcd. for $C_{22}H_{22}O_7N_4S_2$: C, 50.95; H, 4.28; N, 10.81; S, 12.37. Found: C, 49.97; H, 3.81; N, 10.68; S, 12.26.

Attempt to Prepare the Quarternary Salt (XXI)——To a solution of XX (50 mg) in acetonylacetone (1 ml) was added NaOAc or KI (100 mg) and the mixture was heated at 120° for 5 hr.[11] Thin layer chromatography (E. Merck, DC-Alufolien Kieselgel F 254, n-BuOH saturated with H_2O) of the reaction mixture revealed the presence of a major compound, XX and a minor unidentified compound which travelled faster and showed the same UV spectra as those of XX.

9-Imino-3-*β*-D-ribofuranosylthiazolo[3,2-*a*]purine (XXIII)——To a solution of 2-mercaptoadenosine[12] (XXII) (250 mg, 0.84 mmole) in DMF (3 ml) were added *cis*-dichloroethylene (0.1 ml) and 1 N NaOEt (5 ml).

The mixture was heated at 120° for 30 min, then poured into ice-water (10 ml) and adjusted to pH 3. The solution was applied to a column of activated charcoal (3 g). The column was washed with H_2O and eluted with a mixture of EtOH, H_2O, *n*-BuOH and 28% NH_4OH (45: 48: 5: 2 v/v). The eluate was evaporated to dryness *in vacuo*. The residue was recrystallized from water to afford colorless crystals (180 mg). mp 230—232° (decomp.). UV $\lambda_{\max}^{\mathrm{PH1}}$ nm (ε): 244 (3.44×10^4), 290 (1.17×10^4), 303 (1.17×10^4), 310 (sh); $\lambda_{\max}^{\mathrm{PH12}}$ nm (ε): 231, 283 (1.42×10^4), 293 (1.61×10^4), 310 (sh). NMR (d_6-DMSO) δ: 5.80 (1H, d, $J=6$ Hz, H_1'), 7.30 (1H, d, $J=5$ Hz, thiazol ring H), 8.10 (1H, d, $J=5$ Hz, thiazol ring H), 8.16 (1H, s, H_2). *Anal.* Calcd. for $C_{12}H_{13}O_4N_5S\cdot1/2H_2O$: C, 43.37; H, 4.25; N, 21.08; S, 9.65. Found: C, 43.20; H, 4.20; N, 21.10; S, 9.30.

2-Bromoinosine (XXIV)·Ammonium Salt——Compound (I) (2 g) was added to the ice-cooled 47% hydrobromic acid (25 ml). To the vigorously stirred mixture was added bromine (2 ml) dropwise at 0—5°. The reaction mixture was stirred for 2 hr after the addition of bromine. An orange solid precipitated, which was collected by filtration, and washed with cold water. The precipitate was suspended in cold water and the mixture neutralized to pH 7 with 28% NH_4OH to obtain clear solution, which was applied to the column of activated charcoal (20 g). The column was washed with water and eluted with a mixture of EtOH, H_2O, *n*-BuOH and 28% NH_4OH (45: 48: 5: 2 v/v). The eluate was evaporated to dryness. The residue was recrystallized from EtOH to give colorless crystals (1.2 g), mp 176—178° (decomp.). UV $\lambda_{\max}^{\mathrm{PH2}}$ nm (ε): 252 (1.20×10^4); $\lambda_{\max}^{\mathrm{PH12}}$ nm (ε): 257 (1.29×10^4). *Anal.* Calcd. for $C_{10}H_{11}O_5N_4Br\cdot NH_3\cdot1/2H_2O$: C, 32.18; H, 4.05; N, 18.76. Found: C, 32.11; H, 3.90; N, 18.95.

2-Hydrazinoinosine (XXV)——To a solution of XXIV (1 g) in methylcellosolve (20 ml) was added 85% $NH_2NH_2\cdot H_2O$ (2 ml). The mixture was heated at 120° for 3 hr. After cooling, the gel was filtered, washed with water and dried to give white powder (750 mg). UV $\lambda_{\max}^{\mathrm{PH1}}$ nm: 253; $\lambda_{\max}^{\mathrm{PH12}}$ nm: 254, 280 (sh). *Anal.* Calcd. for $C_{10}H_{14}O_5N_6\cdot H_2O$: C, 37.97; H, 5.10; N, 26.57. Found: C, 37.25; H, 4.67; N, 27.37.

3-β-D-Ribofuranosyl-s-triazolo[4,3-*a*]purine-9-one (XXVI) and Its 2′,3′-O-Ethoxymethylidene Derivative (XXVII)——A mixture of XXV (1.4 g), ethyl orthoformate (7 ml) and DMF (50 ml) was heated at 100° for 5 hr. The PE of the reaction mixture revealed the presence of two UV absorbing compounds (the slower travelling compound had a negative color reaction specific for *cis*-OH[13]) and the faster travelling compound had the positive color reaction). The reaction mixture was diluted with water and applied to a column of DEAE cellulose (HCO_3^- form, 100 ml). The column was washed with water and eluted with 0.05 M NH_4HCO_3. The eluate was evaporated to give colorless needles of XXVI (280 mg), mp 237—238°. NMR (d_6-DMSO) δ: 4.94 (1H, double d, H_3'), 5.30 (1H, double d, H_2'), 6.12 (1H, s, $\genfrac{}{}{0pt}{}{-O}{-O}\!\!>\!C\!<\!\genfrac{}{}{0pt}{}{H}{}$), 6.22 (1H, d, $J=3$ Hz, H_1'), 8.19 (1H, s, H_2), 9.02 (1H, s, H_7), 14.1 (1H, broad s, H_{N^5}). *Anal.* Calcd. for $C_{14}H_{16}O_6N_6$: C, 46.14; H, 4.42; N, 23.07. Found: C, 45.75; H, 4.26; N, 22.93. The column was eluted with 0.1 M NH_4HCO_3 and the eluate was evaporated to dryness. The residue was recrystallized from EtOH to give crystals of XXVII (550 mg), mp 249° (decomp.). UV $\lambda_{\max}^{\mathrm{PH2}}$ nm (ε): 216.5 (2.54×10^4), 262 (8.5×10^3), 293 (1.03×10^4); $\lambda_{\max}^{\mathrm{PH12}}$ nm (ε): 224.5 (2.67×10^4), 269 (6.6×10^3), 308 (1.17×10^4).

Acid Hydrolysis of Tricyclic Ribonucleosides——Fifty mg each of XXVI, VIII, III, and IV was dissolved in 0.1 N HCl (5 ml) and the solution was heated in a stoppered glass tube at 100° (boiling water bath). After a definite time (5, 10, 20, 30, 40, and 50 min), the extent of the hydrolysis of the glycosidic linkage was examined by PE (0.05 M borate buffer). All tricyclic ribonucleosides were completely hydrolyzed in 30 min, while guanosine was similarly hydrolyzed in 50 min.

Acknowledgement The authors thank to Drs. S. Tatsuoka and E. Ohmura of this Devision for their encouragement throughout this work. The authors are also grateful to Prof. M. Ikehara, Osaka University, for his useful suggestion.