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Synthesis of Thio-AICA, 6-Thioxanthine, Isoguanine Analogs, and Their Ribosides

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4-Amino-5-thiocarbamoylimidazole (II, thio-AICA) was prepared by direct thiation of 4-amino-5-imidazolecarboxamide (I, AICA) with phosphorus pentasulfide and also by addition of 4-amino-5-imidazole carbonitrile (III) with hydrogen sulfide. Similarly, 5-amino-4-thiocarbamoyl-1-β-D-ribofuranosylimidazole (XVII, thio-AICA-riboside) was prepared by addition of 5-amino-1-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)imidazole-4-carbonitrile (XIII) with hydrogen sulfide followed by removal of the isopropylidene group. XVII was converted by treatment with ethyl formate in ethanolic sodium ethoxide to 6-mercapto-9-β-D-ribofuranosylpurine (XVIII, 6-thioinosine) in good yield and with diethyl carbonate under the similar conditions to 6-mercapto-2-hydroxy-9-β-D-ribofuranosylpurine (XXI, 6-thioxanthosine). Methylation of the latter with methyl iodide and subsequent amination afforded 6-amino-2-hydroxy-9-β-D-ribofuranosylpurine (XXIII, isoguanosine). In a similar fashion, N^6-substituted isoguanosines were prepared by reaction of 6-methylthio-2-hydroxy-9-β-D-ribofuranosylpurine (XXII) with the appropriate amines.

Since the antitumor activity[2] of 6-mercaptopurine (V) had been found biologically and clinically, a number of derivatives of V have been synthesized. Among these compounds, 6-mercapto-9-β-D-ribofuranosylpurine (XVIII, 6-thioinosine),[3] 6-thioguanine,[4] and 6-thioguanosine[3b] were found to exhibit significant activity. Therefore, attention was directed to the synthesis of sulfur-containing derivatives of naturally occurring bases and nucleosides. 2-Mercaptoinosine,[5] an analog of xanthosine, has previously been prepared in our laboratories but did not show antitumor activity.

In this paper the present authors wish to report on the synthesis of 4-amino-5-thiocarbamoylimidazole (II, thio-AICA), 6-mercapto-2-hydroxypurine (VII, 6-thioxanthine), isoguanine analogs, and their ribosides. It is desirable to prepare the analogs of isoguanosine (2-hydroxyadenosine), since adenosine analogs detected in nature, such as Puromycin,[6] Tubercidin,[7] and Toyocamycin,[8] are well known to show significant biological activities. Of particular interest is 5-amino-4-thiocarbamoyl-1-β-D-ribofuranosylimidazole (XVII, thio-AICA-riboside) as a potential antimetabolite because of its structural similarity to 5-amino-1-β-D-ribofuranosyl-4-imidazolecarboxamide (AICA-riboside) playing an important role in purine biosynthesis.

1) Location: *a*) *Suzuki-cho, Kawasaki*; *b*) *Toneyama, Toyonaka, Osaka.*
2) G.H. Hitchings and C.P. Rhodes, *Ann. N. Y. Acad. Sci.*, **60**, 183 (1954).
3) *a*) J.A. Johnson, Jr, and H.J. Thomas, *J. Am. Chem. Soc.*, **78**, 3863 (1956); *b*) J.J. Fox, I. Wempen, A. Hampton, and I.L. Doerr, *J. Am. Chem. Soc.*, **80**, 1669 (1958); *c*) M. Ikehara, *Chem. Pharm. Bull.* (Tokyo), **8**, 367 (1960); *d*) M. Ikehara, H. Uno, and F. Ishikawa, *Chem. Pharm. Bull.* (Tokyo), **12**, 267 (1964).
4) G.B. Elion and G.H. Hitchings, *J. Am. Chem. Soc.*, **77**, 1676 (1955).
5) A. Yamazaki, I. Kumashiro, and T. Takenishi, *J. Org. Chem.*, **32**, 3032 (1967).
6) B.R. Baker, R.E. Shaub, J.P. Joseph, and J.H. Williams, *J. Am. Chem. Soc.*, **77**, 12 (1955).
7) K. Anzai, G. Nakamura, and S. Suzuki, *J. Antibiotics* (Tokyo), **A 10**, 201 (1957).
8) H. Nishimura, K. Katagiri, K. Sato, M. Mayama, and N. Shimaoka, *J. Antibiotics* (Tokyo), **A 9**, 60 (1956).

In 1956, Hitchings, *et al.*[9] first reported that the thiation of 4-amino-5-imidazolecarboxamide (I, AICA) with phosphorus pentasulfide afforded II which was condensed with formamide to give V. Alternatively, Shaw, *et al.*[10] prepared II, isolated as a picrate, by reaction of α-amino-α-cyanothioacetamide with isopentyl formimidate and subsequent ammoniacal treatment. On the other hand, it has previously been shown[11] that II, obtained by thiation of hydrochloride of I, was formylated with formic acid and acetic anhydride and then cyclized by alkaline treatment to hypoxanthine but no V. However, since, in each case, II had not been isolated in a pure state and not well characterized, the original reports have been carefully reinvestigated. Two general methods can be expected to produce II, involving direct thiation of I as already reported and addition of hydrogen sulfide to 4-amino-5-imidazole carbonitrile (III).[12] A successful preparation of II by direct thiation was achieved in the presence of a small amount of water, employing the free base[13] of I. An intermediate thiophosphate ester,[14] which was formed by thiation of I with phosphorus pentasulfide, was readily decomposed in boiling acidic solution to give the hydrochloride of II in 21% yield. The desired compound was also obtained in good yield when III was treated with hydrogen sulfide in the presence of potassium hydroxide. II thus obtained was quite stable to refluxing in 1 N hydrochloric acid for 1 hr and its structure was confirmed by elemental analysis and Grote's reagent,[15] by ultraviolet absorption spectrum which showed maxima at 270.5 and

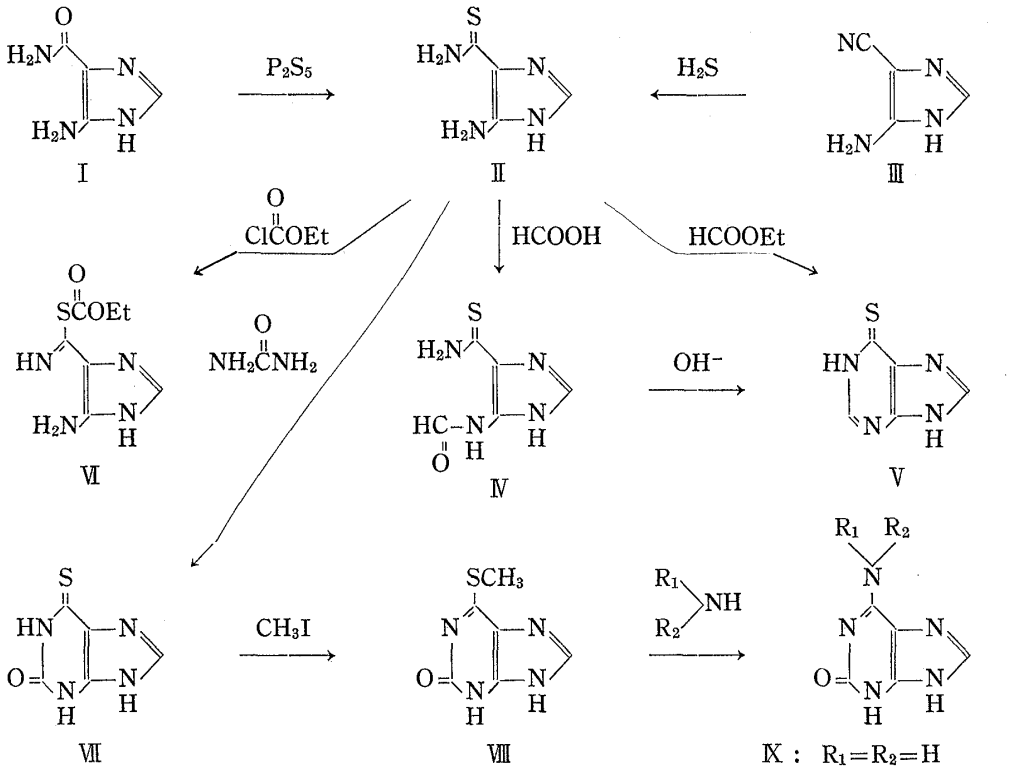


Chart 1

9) G.H. Hitchings and G.B. Elion, U.S. Patent 2756228 (1956).
10) G. Shaw and D.N. Butler, *J. Chem. Soc.*, **1959**, 4040.
11) M. Ikehara, N. Nakazawa, and H. Nakayama, *Chem. Pharm. Bull.* (Tokyo), **10**, 660 (1962).
12) *a*) J.P. Ferris and L.E. Orgel, *J. Am. Chem. Soc.*, **87**, 4976 (1965); *b*) *Idem*, *ibid.*, **88**, 1074 (1966); *c*) *Idem*, *ibid.*, **88**, 3829 (1966); *d*) Y. Yamada, I. Kumashiro, and T. Takenishi, *Bull. Chem. Soc. Japan*, **41**, 241 (1968).
13) A. Yamazaki, I. Kumashiro, and T. Takenishi, *J. Org. Chem.*, **32**, 3258 (1967).
14) F. Bergmann and A. Kalmus, *J. Chem. Soc.*, **1962**, 860.
15) I.W. Grote, *J. Biol. Chem.*, **93**, 25 (1931).

330 mμ at pH 7, and by infrared spectrum which showed a strong band due to the thioamido group at 1468 cm^{-1}.[16] Further support for the structure was obtained by a series of the following experiments.

Formylation of II with formic acid afforded 4-formamido-5-thiocarbamoylimidazole (IV) in 65% yield. Since this compound possesses a structural relationship to the base of purine nucleotide precursor (5-formamido-4-imidazolecarboxamide ribotide), *viz.* 4-formamido-5-imidazolecarboxamide, some biological property as an antimetabolite may be expected for IV. The structure of IV was established by spectral properties; ultraviolet absorption spectrum revealed maxima at 278 and 325 mμ at pH 1, whereas the spectrum in alkaline solution (pH 13) lost completely a maximum at about 280 mμ and was identical with that of V.[17] Thus, IV was unstable toward alkali and could be converted quantitatively to V.[18] The compound V was also prepared by condensation of II with ethyl formate in ethanolic sodium ethoxide in 59% yield according to the method[13] previously reported.

In order to obtain VII, the procedure employed for the preparation of xanthine[19] from I was applied to II. Acylation of II with ethyl chloroformate in pyridine afforded a carbethoxy derivative, which was assumed to be 4-amino-5-(S-carbethoxy)thiocarbamoylimidazole (VI) on the basis of analytical data and chemical reaction. When VI was allowed to reflux in ethanolic sodium ethoxide, no VII was formed and II was partly recovered. Attempt to cyclize II with diethyl carbonate was also unsuccessful. The compound VII was readily prepared by fusion of II with urea in 77% yield and identical in all respects with an authentic sample[20] prepared by thiation of xanthine. Methylation of VII with methyl iodide in alkaline solution gave 6-methylthio-2-hydroxypurine (VIII), which was aminated with ammonia in an autoclave at 130° for 3 hr to yield 6-amino-2-hydroxypurine (IX, isoguanine).[19,21] In a similar manner, 6-methylamino-(X), 6-dimethylamino-(XI), and 6-ethylamino-2-hydroxypurine (XII) were prepared from VIII.

Although there are two reports[22] on the synthesis of sulfur-containing AICA-riboside derivatives, XVII itself has not yet been reported in the literature. Following the similar conditions used for preparing II, 5-amino-4-cyano-1-(2,3-O-isopropylidene-β-D-ribofuranosyl)-imidazole (XIII)[23] was treated with hydrogen sulfide to afford 5-amino-4-thiocarbamoyl-1-(2′, 3′-O-isopropylidene-β-D-ribofuranosyl)imidazole (XIV). This was also prepared by addition[24] of XIII with thioacetic acid followed by treatment of the resulting acetylthio derivative

16) L.J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd ed., Methuen and Co., Ltd., London, 1958, p. 357.

17) Formylation of II with formic acid and acetic anhydride followed by cyclization with alkali was proved to afford the only one product, V, in good yield.

18) G.B. Elion, W.H. Lange, and G.H. Hitchings, *J. Am. Chem. Soc.*, **78**, 2858 (1956); G.B. Elion, E. Burgi, and G.H. Hitchings, *ibid.*, **74**, 411 (1952); A. Bendich, P.J. Russell, and J.J. Fox, *ibid.*, **76**, 6073 (1954).

19) E. Shaw, *J. Biol. Chem.*, **185**, 439 (1950).

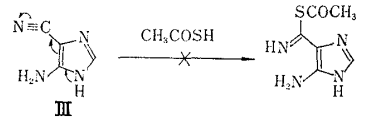
20) A.G. Beaman, *J. Am. Chem. Soc.*, **76**, 5633 (1954).

21) E. Fisher, *Ber.*, **30**, 2226 (1897); A. Bendich, J.F. Tinker, and G.B. Brown, *J. Am. Chem. Soc.*, **70**, 3109 (1948); L.F. Cavalieri, J.F. Tinker, and G.B. Brown, *ibid.*, **71**, 3973 (1949); J. Davoll and B.A. Lowy, *ibid.*, **74**, 1563 (1952); E.C. Taylor, O. Vogl, and C.C. Cheng, *ibid.*, **81**, 2442 (1959); G.B. Brown, G. Levin, and S. Murphy, *Biochemistry*, **3**, 880 (1964).

22) T.L. Perry, S. Hansen, H.P. Bar, and L. MacDougall, *Science*, **152**, 776 (1966); J.A. Montgomery, K. Hewson, S.J. Clayton, and H.J. Thomas, *J. Org. Chem.*, **31**, 2202 (1966).

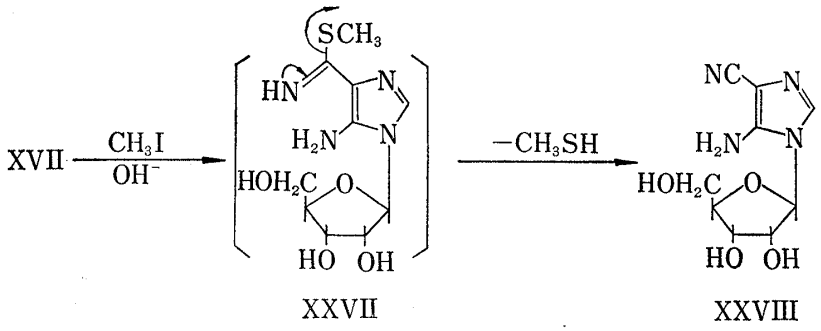
23) This compound was prepared by dehydration of 5-amino-4-carbamoyl-1-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)imidazole with *p*-toluenesulfonyl chloride; Dr. T. Meguro, unpublished work.

24) Addition of III with thioacetic acid did not occur probably because of the decrease of polarity in the nitrile-carbon as follows.



III

(XV) with ammonia. Although XV was chromatographically homogeneous, difficulty was encountered in obtaining it as a pure crystal. The compound XIV was then hydrolyzed to XVII,[25] which showed characteristic absorption in the ultraviolet at 330 mμ and possessed some chemically interesting properties. Attempts to prepare aminomethyl derivative (XX) by desulfurization of XVII with Raney nickelwere unsuccessful and, moreover, methylation of XVII with methyl iodide in alkaline solution failed to give S-methyl derivative (XXVII); in this case, the elimination of methyl mercaptan occurred as shown below and the cyano derivative (XXVIII) was regenerated. It is noteworthy that, since XVII is considerably hydrolyzed to AICA-riboside[26] by refluxing in 1 N sodium hydroxide, the thiocarbamoyl group is rather susceptible to alkali.



XXVII XXVIII

Chart 3

When XVII was allowed to react with ethyl formate in the presence of sodium ethoxide, XVIII was obtained in 77% yield. This simple procedure provided a new synthetic route to XVIII which had been prepared by thiation[3b,c] of inosine derivative or by reaction[2] of 6-chloropurine riboside derivative with thiourea. Similar treatment of XIV with the ester furnished 6-mercapto-9-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)purine (XVI)[27] in 64% yield Alternatively, this was obtained by condensation of XV with ethyl formate, in which the ring closure proceeded *via* XIV in the presence of sodium ethoxide. Recent investigations demonstrated that glutamine ribosylpyrophosphate 5-phosphate amidotransferase was inhibited[28] significantly by 6-mercapto-9-β-D-ribofuranosylpurine 5′-phosphate and that the latter was converted in part to the 5′-nucleotide[29] of formylaminoimidazolethioamide during the preparation of crystalline pyridinium nucleotide. These facts prompted us to the synthesis of 5-amino-4-thiocarbamoyl-1-β-D-ribofuranosylimidazole 5′-phosphate (XIX). Compound XIV was then phosphorylated with phosphoryl chloride in trimethyl phosphate[30] followed by removal of the isopropylidene group to afford XIX, which was isolated as pure crystals though in low yield.

The preparation of 6-mercapto-2-hydroxy-9-β-D-ribofuranosylpurine (XXI, 6-thioxanthosine) was accomplished by reaction of XVII with diethyl carbonate. Subsequent methylation of XXI with methyl iodide resulted in 6-methylthio-2-hydroxy-9-β-D-ribofuranosylpurine (XXII) in 54% yield. It is of interest to note that both alkaline and acidic solutions of XXII

25) An alternative preparation of XVII was accomplished by direct thiation of 5-amino-4-carbamoyl-1-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl)imidazole with phosphorus pentasulfide followed by deacetylation; Dr. K. Suzuki, unpublished work.
26) T. Shiro, A. Yamanoi, S. Konishi, S. Okumura, and M. Takahashi, *Agr. Biol. Chem.* (Tokyo), **26**, 785 (1962).
27) A. Hampton, *J. Am. Chem. Soc.*, **83**, 3640 (1961).
28) R. J. McCollister, W.R Gilbert, Jr., D.M. Ashton, and J.B. Wyngaarden, *J. Biol. Chem.*, **239**, 1560 (1964).
29) M.R.Atkinson, J.F. Jackson, R.K. Morton, and A.W. Murray, *Nature*, **196**, 35 (1962).
30) M. Yoshikawa, T. Kato, and T. Takenishi, *Tetrahedron Letters*, **1967**, 5065.

Table

Compd.	mp (°C)	*Rf*[a] A	*Rf*[a] B	$[\alpha]_D$	UV λ_{max} mμ (ε) pH 1	pH 7	pH 13
II	>260	0.60	0.50		280 (9500), 327 (14700)	270.5 (9500), 330 (14000)	271.5 (11800), 343 (16900)
IV	>260		0.53		278 (10000), 325 (12000)	274 (10800), 325 (12200)	.....
VI	>260	0.61	0.50		273 (10400), 330 (11600)	276.5[c] (11400), 331 (11600)	272 (10600), 340 (15200)
VIII	>260	0.62	0.47		265[b] (6600), 335 (12600)	266 (8100), 318 (11800)	320[b] (10100)
X	>260	0.57	0.38		287 (13000)	286 (8900)	287 (13900)
XI	>260	0.59	0.37		292.5 (13400)	251 (10700), 288 (11900)	290 (12400)
XII	>260	0.64	0.49		288 (14000)	245 (8200), 284 (10400)	288 (14400)
XIV	75—76	0.85	0.76	$[\alpha]_D^{25}$ −102.1° (c=1, EtOH)	281.5 (9000), 327 (12900)	272 (9700), 329 (13400)	272 (4600), 330 (10200)
XIX	168 (decomp.)	0.25	0.08	$[\alpha]_D^{26}$ −100.4° (c=0.74, 0.1 N NaOH)	282 (7700), 327 (12400)	272 (8300), 329.5 (11800)	272 (8400), 331.5 (11900)
XVII	143	0.65	0.36	$[\alpha]_D^{17}$ −92.5° (c=2, 1 N NaOH)	282 (8900), 327 (15400)	273 (10700), 330 (13100)	271.5 (10200), 330 (16500)
XXI	150 (decomp.)	0.54	0.20	$[\alpha]_D^{18}$ −69.0° (c=2, 0.1 N NaOH)	257 (6900), 337 (22500)	256 (9400), 343 (25200)	255 (8700), 343 (15400)
XXII	163	0.59	0.42	$[\alpha]_D^{27}$ −67.0°[b] (c=2, 1 N NaOH)	254[b] (6500), 333 (12100)	244 (13100), 315.5 (12600)	243[b] (13300), 313 (13100)
XXIV	248 (decomp.)	0.49	0.25	$[\alpha]_D^{26}$ −63.1° (c=0.09, 0.1 N NaOH)	285 (15400)	250.5 (9700), 297 (11400)	287 (12300)
XXV	220 (decomp.)	0.58	0.29	$[\alpha]_D^{26}$ −52.0° (c=0.07, 0.1 N NaOH)	289 (15700)	254 (10200), 304 (11800)	290 (13500)
XXVI	212 (decomp.)	0.61	0.39	$[\alpha]_D^{26}$ −68.2° (c=0.97, 0.1 N NaOH)	287 (15200)	251 (9700), 299 (11800)	288 (12100)
XXIX	165—166	0.74	0.59	$[\alpha]_D^{25}$ −60.9° (c=1, 0.1 N NaOH)	257 (6400), 337 (18300)	255 (7600), 343.5 (18500)	255 (9500), 342 (14000)
XXX	187—188	0.79	0.80	$[\alpha]_D^{25}$ −110.8° (c=1, $CHCl_3$)	252[b] (6900), 333.5 (11500)	244 (8100), 315 (11900)	244[b] (14000), 314 (13400)
XXXI	290 (decomp.)	0.55	0.55	$[\alpha]_D^{25}$ −61.4° (c=1, 0.1 N NaOH)	237 (5800), 284 (13300)	250 (8000), 296 (10300)	254 (7200), 286 (10800)
XXXII	253—254 (decomp.)	0.75	0.72	$[\alpha]_D^{25}$ −85.5° (c=1, 0.1 N NaOH)	287 (16400)	254 (9900), 299 (13600)	289 (13800)
XXXIII	133—135 (decomp.)	0.78	0.76	$[\alpha]_D^{25}$ −65.7° (c=1, 0.1 N NaOH)	286 (15800)	251 (8700), 297 (13100)	288 (14900)

a) All chromatographies were performed on Toyo Filter Paper No. 51 by the ascending technique. solvent systems: A, *n*-propyl alcohol-ammonia (28%)-water, 20:12:3 v/v; B, *n*-butyl alcohol-acetic acid-water, 4:1:1 v/v; C, isopropyl alcohol-sat. ammonium sulfate-water, 2:79:19 v/v

showed[31] a considerable decrease in optical density and a marked change in ultraviolet absorption spectra after several hours at room temperature. This may be due to the decomposition of purine nucleus and degradation products were not further investigated.

When XXII was aminated with ammonia, 6-amino-2-hydroxy-9-β-D-ribofuranosylpurine (XXIII, isoguanosine),[32] namely, Crotonoside which occurred in nature, was readily obtained.

31) Also in cases of XVIII and thioguanosine, a similar decrease in optical density has been observed by Fox, *et al.*[3b]

32) E. Cherbuliez and K. Bernhart, *Helv. Chim. Acta*, **15**, 464 (1932); F. Schlenk, M.L. Schaedel, and M. J. Waldvogel, *J. Biol. Chem.*, **171**, 135 (1947); J. Davoll, *J. Am. Chem. Soc.*, **73**, 3174 (1954).

I

Formula	Analysis (%) Calcd. C	H	N	P	Found C	H	N	P
$C_4H_7N_4SCl$	26.89	3.95	31.36		27.15	4.36	31.85	
$C_5H_6ON_4S$	35.30	3.55	32.93		35.57	4.10	33.51	
$C_7H_{10}O_2N_4S$	39.25	4.71	26.16		39.37	4.91	26.46	
$C_6H_6ON_4S$	39.56	3.32	20.76		39.40	3.52	30.94	
$C_6H_7ON_5$	43.36	4.27	42.41		43.22	4.50	41.85	
$C_7H_9ON_5 \cdot \frac{1}{2}H_2O$	44.63	5.31	37.19		44.63	5.45	37.63	
$C_7H_9ON_5$	46.92	5.06	39.09		46.63	5.29	39.29	
$C_{12}H_{18}O_4N_4S \cdot CHCl_3$	35.97	4.42	12.92		36.25	4.59	12.96	
$C_9H_{15}O_7N_4PS$	29.75	4.45	15.42	8.76	29.96	4.52	15.59	8.53
$C_9H_{14}O_4N_4S$	39.40	5.14	20.43		39.18	5.12	20.16	
$C_{10}H_{12}O_5N_4S \cdot \frac{3}{4}H_2O$	38.27	4.35	17.86		38.74	4.61	17.33	
$C_{11}H_{14}O_5N_4S \cdot \frac{1}{2}H_2O$	40.90	4.69	17.35		41.14	4.56	17.46	
$C_{11}H_{15}O_5N_5$	44.44	5.09	23.56		44.94	5.67	23.21	
$C_{12}H_{17}O_5N_5$	46.29	5.51	22.50		46.09	5.78	22.35	
$C_{12}H_{17}O_5N_5$	46.29	5.51	22.50		46.13	5.86	22.87	
$C_{13}H_{16}O_5N_4S \cdot H_2O$	43.58	5.07	15.63		43.85	4.90	16.04	
$C_{14}H_{18}O_5N_4S$	47.16	5.32	15.87		47.45	5.12	15.81	
$C_{13}H_{17}O_5N_5 \cdot \frac{1}{2}H_2O$	46.99	5.42	21.08		47.03	5.82	21.11	
$C_{15}H_{21}O_5N_5 \cdot H_2O$	48.78	6.23	18.97		48.66	6.23	19.05	
$C_{15}H_{21}O_5N_5 \cdot H_2O$	48.78	6.23	18.97		48.85	6.65	19.33	

b) As soon as the sample was dissolved, ultraviolet absorption spectrum and optical density were measured.
c) Measured in ethanol.

The N^6-substituted isoguanosines (XXIV—XXVI), with the results listed in Table I, were prepared by allowing XXII to react with the appropriate amines.

Some isopropylidene derivatives, which were direct precursors for 5′-nucleotides, were synthesized. Compound XIV reacted with diethyl carbonate to yield the isopropylidene derivative (XXIX) of XXI, which was methylated as usual to afford S-methylthio derivative (XXX). Ammonolysis of XXX provided 6-amino-2-hydroxy-9-(2′,3′-O-isopropylidene-9-β-D-ribofuranosyl)purine (XXXI). Likewise, reaction of XXX with dimethylamine and ethylamine gave the corresponding isopropylidene derivatives (XXXII and XXXIII), respectively.

A detailed result of antitumor activity of the synthesized compounds will be reported later.

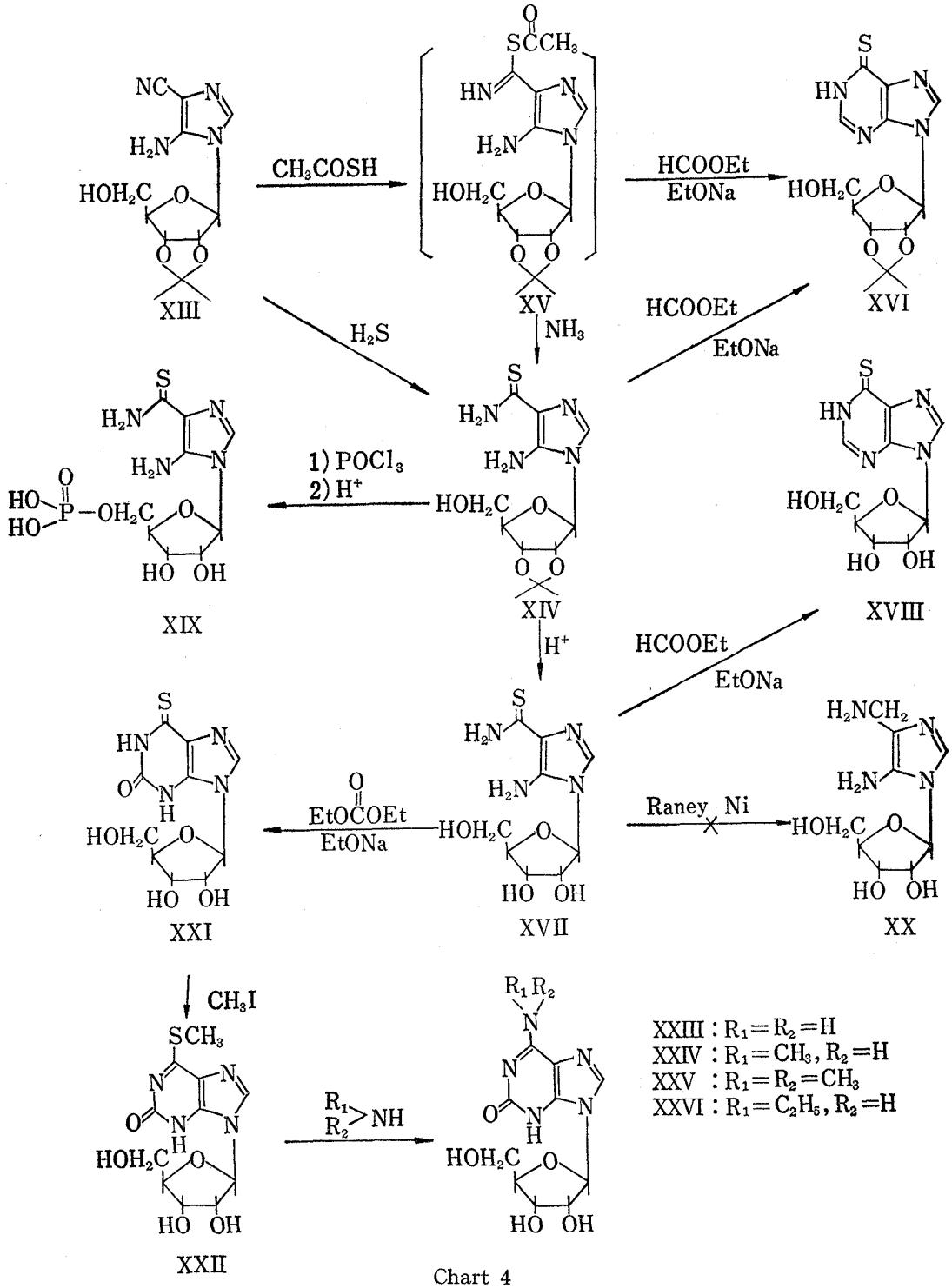


Chart 4

Experimental[33]

4-Amino-5-thiocarbamoylimidazole Hydrochloride (II, Thio-AICA) A——A solution of the free base[13] of 4-amino-5-imidazolecarboxamide (50 g, 0.397 moles) in 1 liter of pyridine was stirred and refluxed. There was added 140 g of P_2S_5 portionwise and carefully. After adding a half of the reagent, a vigorous

33) All melting points are uncorrected. Ultraviolet absorption spectra were taken with a Hitachi EPS-3T automatic recording spectrophotometer, and infrared absorption spectra were measured with a Jasco Model IR-S spectrophotometer. The nuclear magnetic resonance spectrum was measured with a Varian A-60 using tetramethylsilane as internal standard.

reaction occurred and yellow crystals precipitated. The residual reagent and 1 ml of H_2O were added and stirring was continued for 2 hr. At the end of the reaction, the solution turned to dark brown. The resulting brown solid or gum, thiophosphate ester, was collected by filtration and dissolved in 300 ml of 3 N HCl. After being refluxed for 30 min to decompose completely the phosphorus compound, the solution was concentrated *in vacuo* to a gummy product, which was taken up in 50 ml of H_2O. Cooling in a refrigerator afforded yellow crystals, which were recrystallized from a small amount of H_2O to give 15 g (21.2%) of yellow crystals.

B——To a solution of 4-amino-5-imidazole carbonitrile hydrochloride[12d] (III, 2 g, 13.8 mmoles) in 360 ml of MeOH, a solution of KOH (7.7 g, 138 mmoles) in 50 ml of MeOH was added and the mixture was saturated with H_2S at 0°, during which K_2S precipitated. The mixture was heated in an autoclave at 100° for 2 hr, concentrated to about 50 ml after addition of H_2O (300 ml), and acidified with AcOH. The resulting precipitate was filtered and dissolved in 200 ml of 1 N HCl. Concentration of the solution under reduced pressure afforded yellow crystals, which were recrystallized from H_2O with charcoal to afford 1.5 g (61%) of analytically pure yellow crystals identical with the sample prepared in A. This compound gave an orange color with Pauly's test[34] and a deep green color on slight warming with Grote's reagent.[15]

5-Amino-4-thiocarbamoyl-1-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)imidazole (XIV) **A**——5-Amino-4-cyano-1-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)imidazole[23] (XIII, 10 g, 35.7 mmoles) and KOH (4 g, 71.4 mmoles) were dissolved in 100 ml of MeOH. The solution was then saturated with H_2S at 0° and heated in an autoclave at 100° for 3 hr. After H_2O (100 ml) was added, the mixture was concentrated under reduced pressure to *ca.* 50 ml and the product was extracted with three 100 ml portions of $CHCl_3$. The combined extracts were dried over anhydrous Na_2SO_4 and evaporated *in vacuo* to dryness. The yellowish residue (7 g) was suitable for use in next reaction. Crystallization from $CHCl_3$ yielded 2.3 g (14.8%) of light yellow needles having the molecular formula $C_{12}H_{18}O_4N_4S \cdot CHCl_3$. The nuclear magnetic resonance spectrum in hexadeuteriodimethyl sulfoxide indicated a singlet at 8.25 ppm due to the proton of $CHCl_3$ and the proton signal at 2-position appeared at 7.74 ppm (singlet).

The picrate was prepared in EtOH and recrystallized from the same solvent to give a pure sample, mp 194—195° (decomp.). *Anal.* Calcd for $C_{18}H_{21}O_{11}N_7S$: C, 39.78; H, 3.90; N, 18.05. Found: C, 39.25; H, 4.36; N, 18.25.

B——After a gummy XV (3 g) was dissolved in 20 ml of H_2O, the solution was saturated with NH_3 at 0°, allowed to stand in a refrigerator overnight, and concentrated under reduced pressure. The residue was dissolved in 10 ml of H_2O and the product was extracted with $CHCl_3$. Concentration of the extracts gave a yellowish powder (1.8 g) of XIV which showed a single spot on a paper chromatogram.

5-Amino-4-thiocarbamoyl-1-β-D-ribofuranosylimidazole (XVII, Thio-AICA-riboside)——Crude XIV, prepared by addition of XIII (5 g) with H_2S, was dissolved in a mixture of EtOH (20 ml) and H_2O (150 ml). The solution was brought with 1 N HCl to pH 1.5, heated at 70° for 40 min with stirring to remove the isopropyridene group, and neutralized to pH 7 by adding portionwise Amberlite IRA-410 (OH^- form). The resin was removed by filtration and washed with H_2O. The filtrate and washings were combined and concentrated *in vacuo* to precipitate the product. Crystallization from EtOH gave 3.1 g (63.3%) of pure light yellow crystals. The infrared absorption spectrum of this compound showed a strong band at 1478 cm^{-1} for the thioamido group.

5-Amino-4-(S-acetyl)thiocarbamoyl-1-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)imidazole (XV)——A solution of XIII (5 g) in 50 ml of AcSH was refluxed for 30 min and the reaction mixture was then concentrated under reduced pressure to yield a crude XV. This was suspended in 20 ml of H_2O and the product was extracted with three 50 ml portions of $CHCl_3$. Concentration of the extracts *in vacuo* afforded a gummy product (5.1 g), which was homogeneous on a paper chromatogram but failed to crystallize in spite of many efforts. Ultraviolet absorption properties; λ_{max}^{PHI} 280 and 325.5 mμ.

4-Formamido-5-thiocarbamoylimidazole (IV)——The hydrochloride of II (1.78 g, 10 mmoles) was refluxed with HCOONa (0.68 g, 10 mmoles) in 50 ml of HCOOH for 2 hr. Removal of the solvent gave a crystalline product. Recrystallization from aqueous EtOH afforded pure orange crystals, yield 1.1 g (64.7%).

6-Mercaptopurine (V) **A**——Two grams of IV was added to 100 ml of 0.5 N NaOH and the mixture was refluxed for 10 min. This was neutralized by adding Amberlite IR-120 (H^+ form). The resin was removed by filtration, the filtrate was concentrated *in vacuo* to dryness, and the residue was crystallized from H_2O to furnish 1.45 g (81%) of pure yellow crystals. This compound was confirmed to be identical with an authentic sample by comparison of their ultraviolet and infrared absorption spectra.

B——To a solution of ethanolic EtONa (prepared from 3.5 g (152 mg-atoms) of Na and 70 ml of EtOH) was added the hydrochloride of II (2 g, 11.2 mmoles) with stirring. Immediately NaCl precipitated. Ethyl formate (8.2 g, 112 moles) was then added and the mixture was refluxed for 1.5 hr. After 100 ml of H_2O was added, the clear solution was treated batchwise with Amberlite IR-120 (H^+ form). The filtrate, after

34) B.N. Ames and H.K. Mitchell, *J. Am. Chem. Soc.*, 74, 252 (1952).

removal of the resin, was concentrated *in vacuo* to give a crystalline product, which was recrystallized from H_2O, yielding 1.3 g (76%) of yellow crystals.

6-Mercapto-9-β-D-ribofuranosylpurine (XVIII)——The same procedure as described for the method B in V was applied to 5 g of XVII, using 13.3 g of ethyl formate. The crude product was recrystallized twice from H_2O to give light yellow crystals, yield 4 g (77%). This compound was identical with an authentic sample.[3]

6-Mercapto-9-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)purine (XVI) A——A solution of XIV (1 g) in ethanolic EtONa was treated with ethyl formate as described above to obtain a crude product. Recrystallization from H_2O afforded 480 mg (64%) of pure crystals. This compound was identical with an authentic sample[27] by comparison of ultraviolet and infrared absorption spectra.

B——The crude XV (1 g) was treated with ethyl formate as described above. Crystallization of a crude product from H_2O gave 0.25 g (27.5%) of an analytically pure sample.

4-Amino-5-(S-carbethoxy)thiocarbamoylimidazole (VI)——The hydrochloride of II (3.6 g, 20.2 mmoles) was dissolved in 200 ml of pyridine, and to this was added 2.6 g (23.3 mmoles) of ethyl chloroformate. The mixture was allowed to stand at room temperature for 2 days and poured into 500 ml of ice water. The resulting crystals were collected by filtration and recrystallized from H_2O to yield 1.8 g (41.7%) of a product.

6-Mercapto-2-hydroxypurine (VII)——Five grams (28 mmoles) of II hydrochloride and 10 g (166 mmoles) of urea were fused in an oil bath at 150—160° for 1.5 hr. NH_3 gas evolved and the mixture solidified at the end of the reaction. Purification of the solid was accomplished by reprecipitation from 0.5 N NaOH with dilute AcOH. Tan powder (3.65 g, 77.6%) was obtained. This compound was identified by direct comparison of ultraviolet and infrared spectra with those of an authentic sample.[20]

6-Mercapto-2-hydroxy-9-β-D-ribofuranosylpurine (XXI)——XVII (5 g, 18.5 mmoles) was dissolved in a solution of ethanolic EtONa (prepared from 3 g (130 mg-atoms) of Na and 100 ml of EtOH), and to this was added 11 g (93 mmoles) of diethyl carbonate with stirring. The mixture was then refluxed for 3 hr. After H_2O (100 ml) was added, the clear solution was neutralized with 1 N HCl and stored in a refrigerator overnight. The resulting crystals were collected by filtration and recrystallized from H_2O to give 3.1 g (54%) of yellow crystals.

6-Mercapto-2-hydroxy-9-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)purine (XXIX)——A solution of the crude XIV (5 g) in ethanolic EtONa was treated with diethyl carbonate (18.8 g, 159 mmoles) as described above. After reaction, H_2O (500 ml) was added and the pH of the solution was adjusted with Amberlite IR-120 (H^+ form) to 4 and then with dilute NH_4OH to 8. Concentration of the solution gave a crude product (4 g), which was recrystallized from H_2O to afford 2.1 g (37%) of yellow crystals.

6-Methylthio-2-hydroxypurine (VIII)——To a stirred solution of VII (2 g, 11.9 mmoles) in 20 ml of 1 N NaOH was added CH_3I (2.2 g, 15.5 mmoles) portionwise. After being stirred for 2 hr at room temperature, the mixture was acidified with AcOH. The resulting precipitate was filtered and recrystallized from methyl cellosolve, giving 1.3 g (60%) of light yellow product.

6-Methylthio-2-hydroxy-9-β-D-ribofuranosylpurine (XXII)——To a solution of XXI (5 g, 16 mmoles) in 165 ml of 0.2 N NaOH, CH_3I (1.78 g, 12.5 mmoles) was added portionwise with stirring at room temperature. After 1 hr, an additional CH_3I (1.78 g) was added. Stirring was continued for 2 hr after the addition. The solution was then brought with dilute HCl to pH 7 and concentrated *in vacuo* to dryness. The product was extracted several times with hot EtOH. Concentration of the extracts gave a gummy product, which was crystallized from a small amount of H_2O to afford 3.2 g (62%) of XXII as white crystals.

6-Methylthio-2-hydroxy-9-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)purine (XXX)——The compound XXIX (4 g, 11.2 mmoles) was treated with CH_3I (2.05 g, 14.4 mmoles) as described above. After being neutralized with AcOH, extraction of the solution with $CHCl_3$ (3×50 ml) and subsequent evaporation of the extract afforded a crude product. Crystallization from H_2O yielded light yellow crystals. Yield 2.1 g (53%).

6-Amino-2-hydroxypurine (IX)——After compound VIII (1.5 g) was added to 25 ml of concentrated NH_4OH, the solution was saturated with NH_3 at 0° and heated in an autoclave at 130—140° for 3 hr. Concentration of the reaction mixture gave a crude product, which was purified by two reprecipitation from NH_4OH with dilute AcOH. Yield 0.95 g (76.5%). This compound was confirmed to be identical with an authentic IX by comparison of their physical properties.

6-Methylamino-2-hydroxypurine (X)——A sample of VIII (1.5 g) in 18 ml of 30% methylamine was heated in an autoclave at 130—140° for 3 hr. The solvent was removed *in vacuo* and the residue was purified by reprecipitation. Yield 0.72 g (53%).

The following compounds were obtained from 1.5 g of VIII by the same procedure as described for X.

6-Dimethylamino-2-hydroxypurine (XI)——Yield 0.5 g (32.2%).

6-Ethylamino-2-hydroxypurine (XII)——Yield 0.65 g (44%).

6-Amino-2-hydroxy-9-β-D-ribofuranosylpurine (XXIII)——Compound XXII (1 g) was added to 60 ml of EtOH saturated with NH_3 at 0° and the mixture was heated in an autoclave at 120° for 2 hr. The solvent was removed *in vacuo* and the residue was crystallized from H_2O, giving 0.7 g (79.6%) of colorless crystals.

N^6–Substituted isoguanosines and their isopropylidene derivatives were prepared from XXII and XXX, respectively, as described above. All the crude products were crystallized from H_2O.

6-Methylamino-2-hydroxy-9-β-D-ribofuranosylpurine (XXIV)——Yield 53%.

6-Dimethylamino-2-hydroxy-9-β-D-ribofuranosylpurine (XXV)——Yield 40.5%.

6-Ethylamino-2-hydroxy-9-β-D-ribofuranosylpurine (XXVI)——Yield 56%.

6-Amino-2-hydroxy-9-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)purine (XXXI)——Yield 32.4%.

6-Dimethylamino-2-hydroxy-9-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)purine (XXXII)——Yield 28%.

6-Ethylamino-2-hydroxy-9-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)purine (XXXIII)——Yield 39%.

5-Amino-4-thiocarbamoyl-1-β-D-ribofuranosylimidazole 5′-Phosphate (XIX)——A mixture of 50 ml of trimethyl phosphate and 5 g (15.8 mmoles) of the powdered XIV was placed in a 100 ml three-necked flask equipped with a mechanical stirrer, thermometer, and silica gel drying tube. There was added $POCl_3$ (7.3 g, 47.5 mmoles) with stirring while maintaining the temperature below $-5°$ and the mixture was stirred at $-5°$ for 3 hr. The solution was then poured into stirred ice water (500 ml) to decompose the excess of $POCl_3$, brought with 2 N NaOH to pH 1.5, and heated at 70° for 40 min to remove the isopropylidene group. After cooling, the pH was adjusted to 2 and the solution was passed through a column (3×100 cm) of decolorizing resin.[35] The column was washed with H_2O, the nucleotide was eluted with 0.5 N NH_4OH until eluate became free from ultraviolet absorbing material. The eluate was concentrated *in vacuo* to dryness. The residue was dissolved in 300 ml of H_2O and applied to a column (1.5×60 cm) of Dowex 1-XI ($HCOO^-$ form, 50—100 mesh). The column was washed with 3 liters of H_2O, the nucleotide was eluted with 4.2 liters of 0.5 M HCOOH, and 300 ml fractions were collected. The desired product XIX emerged in tubes 7—14. These fractions showed a single spot with *Rf* value of 0.25 on a paper chromatogram in solvent C and were concentrated at 30—40° with a rotary evaporator. The residue was triturated with a small amount of H_2O, and the resulting crystals were collected by filtration. Further elution with 1 M HCOOH (2 liters) and evaporation of the eluate gave a product, which was crystallized as described above. The combined samples were dried *in vacuo* over P_2O_5 at 80° for 2 hr, giving 800 mg (14.4%)[37] of light yellow crystals; the migrating distance in paper electrophoresis (10% AcOH buffer, 800 V/cm, 3 hr): 4.3 cm.

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35) This decolorizing resin was prepared in our laboratories by co-polymerization of metaphenylenediamine, resorcin, and formalin.[36]

36) Y. Tsuchiya, I. Hayashi, T. Kato, M. Yoshikawa, T. Mori, and S. Miyasaka, Japan. Patent 12343 (1964).

37) In view of the alkaline susceptibility of IV, it seems to be quite probable that ring closure of the 5′-nucleotide of formylaminoimidazolethioamide with dilute alkali afforded 6-mercapto-9-β-D-ribofuranosylpurine 5′-phosphate, as suggested by Atkinson, *et al.*[29] However, it should be noted that a considerable difference was observed in the comparison of ultraviolet absorption maxima (at pH 7) of XIX and the above formyl derivative of imidazole nucleotide. The spectrum of the latter is reported to show maximum only at 280 mμ at pH 7 but there is no characteristic absorption due to the thiono group at about 330 mμ, whereas XIX shows maxima at 273 and 329.5 mμ.