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Synthesis of Compounds Related to Antitumor Agents. II.[1] Studies on the Synthesis of Oxazolo[4,5-*d*]pyrimidine Nucleoside Derivatives

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2,4-Diamino-6-methylpyrimidine (II) was synthesized from acetoacetonitrile (III) and guanidine which was then converted to 5-amino-7-methyloxazolo[4,5-*d*]pyrimidin-2(3H)-one (IX). Several nucleoside type compounds were synthesized by the condensation of IX with some halogenoacetyl and halogenobenzoyl sugars in the presence of metal salts as a halogen acceptor. The antitumor activity of the compounds was tested and found to be inactive.

In a recent report, we described the synthesis of new heterocyclic compounds, oxazolo-[4,5-*d*]pyrimidine and pyrimido[5,4-*b*][1,4]oxazine derivatives. This paper describes the syntheses of 2,4-diamino-6-methylpyrimidine (II) as an intermediate in an alternative route and of oxazolo[4,5-*d*]pyrimidine nucleoside derivatives, which were tested for the survival percentages on *Yoshida sarcoma*.

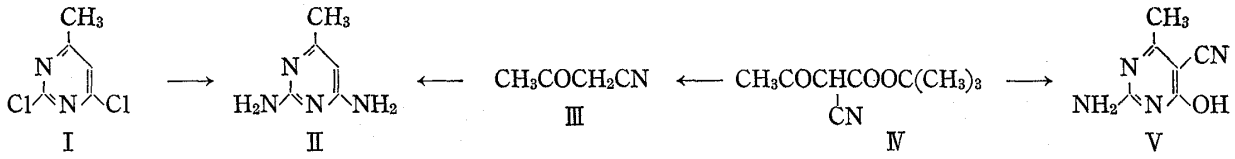


Chart 1

The compound (II) has been synthesized from 2,4-dichloro-6-methylpyrimidine (I) with ethanolic ammonia by Marshall, *et al.*[3] In this method, we found that II was contaminated with monoamino compound which could not readily be separated. Therefore, an alternative synthesis of II was effected using acetoacetonitrile (III)[4] and guanidine in the presence of sodium methoxide. The product (II) was identified with an authentic sample[3] by infrared (IR) spectrum and mixed melting point determination. In the condensation of tert-butyl-α-cyanoacetoacetate (IV)[4] and guanidine, the obtained product was not II but 2-amino-4-hydroxy-6-methyl-5-pyrimidinecarbonitrile (V). The IR spectrum of V exhibited a sharp absorption peak characteristic of cyano group at 2200 cm^{-1} and ultraviolet (UV) spectrum in ethanol showed a maximum absorption peak characteristic of pyrimidine at 260 mμ, and V was identified with an authentic sample[5] by melting point determination. Thus, it is apparent that the Claisen condensation took place at the carbonyl group and not the cyano group. The compound (II) was converted to oxazolo[4,5-*d*]pyrimidine (IX) by way of the previous report.[1a]

1) *a*) Part I: N. Oda, Y. Kanie, and I. Ito, *Yakugaku Zasshi*, 93, 817 (1973); *b*) Presented to the 94th Annual Meeting of the Pharmaceutical Society of Japan, Sendai, April 1974.
2) Location: *a*) *Tanabe-dori, Mizuho-ku, Nagoya*; *b*) *Kanakoden, Chikusa-ku, Nagoya*.
3) J.R. Marshall and J. Walker, *J. Chem. Soc.*, **1951**, 1014.
4) K. Sato and T. Amakatsu, *J. Org. Chem.*, 33, 2446 (1968).
5) C.S. Hee, *Daehan, Hwakak, Hwoejce*, 13, 177 (1969); [*Chem. Abstr.*, 73, 58549[j] (1970)].

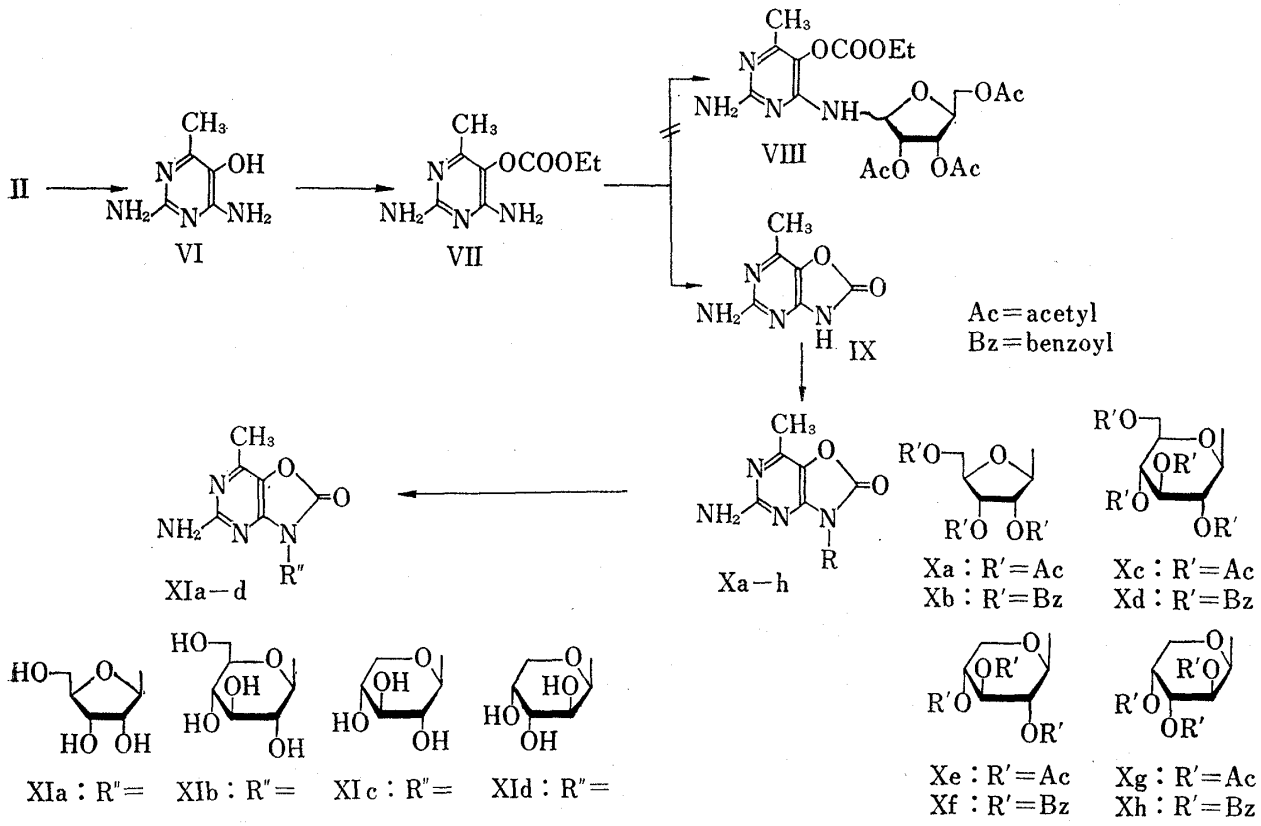
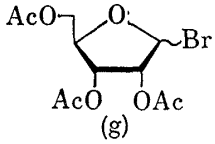


Chart 2

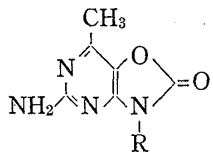
In the condensation of IX with sugars, we tried the following methods; 1) in the presence of alcoholate, 2) in the presence of heavy metal salts[7] and $CaSO_4$, for 1-bromo-2,3,5-tri-O-acetyl-D-ribofuranose[6] and 3) fusion method, for tetra-O-acetyl-β-D-ribofuranose[8] or 1-O-acetyl-tri-O-benzoyl-β-D-ribofuranose.[8] In method 1), the mixture was refluxed for several hours with alcoholate to give Xa in low yields. In method 2) heavy metal salts, $Hg(CN)_2$, $HgCl_2$ or CH_3COOAg were used to compare the efficiency of the heavy metal salts.

Table I. Condition of Reaction in Synthesis of Xa

IX (g)	 (g)	Heavy metal salt (g)	Drierite ($CaSO_4$) (g)	Refluxed time (hr)	Yield (%)	Solvent (ml)
0.332	0.678	$Hg(CN)_2$	1	1	85	CH_3NO_2 (200)
0.332	0.678		—	1	80	
0.498	1.017		1.5	1.5	62	CH_3NO_2 (300)
0.498	1.017		—	1.5	60	
0.332	0.678	$HgCl_2$	1	1	78	CH_3NO_2 (200)
0.332	0.678		—	1	75	
0.498	1.017		1.5	1.5	62	CH_3NO_2 (300)
0.498	1.017		—	1.5	60	
0.332	0.678	CH_3COOAg	1	1	52	CH_3NO_2 (200)
0.332	0.678		—	1	51	
0.498	1.017		1.5	1.5	46	CH_3NO_2 (300)
0.498	1.017		—	1.5	45	

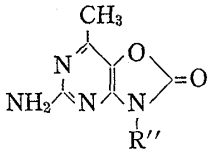
6) J. Davoll, B. Lythoge, and A.R. Todd, *J. Chem. Soc.*, **1948**, 967.
7) N. Yamaoka, K. Aso, and K. Matsuda, *J. Org. Chem.*, **30**, 149 (1965).
8) E.F. Recond and H. Rinderknecht, *Helv. Chim. Acta.*, **42**, 1171 (1959).

As shown in Table I $Hg(CN)_2$ was found to be most appropriate, and $CaSO_4$ had little effect on the yields. Taking this result into consideration, halogenoacetyl and halogenobenzoyl derivatives[6] of glucopyranose, arabopyranose and xylopyranose were condensed as sugar moiety with IX. The properties of the compounds obtained are summarized in Table II.

Table II.  Xa—h

	mp (°C)	Appearance	Yield (%)	Formula	Analysis (%) Calcd. C	Calcd. H	Found C	Found H	Mass (M^+)
Xa	165—169	colorless prisms	80	$C_{17}H_{20}O_9N_4$	48.11	4.75	48.33	5.00	424
Xb	172—174	colorless prisms	85	$C_{32}H_{26}O_9N_4$	62.95	4.29	63.22	4.35	610
Xc	181—183	colorless prisms	66	$C_{20}H_{24}O_{11}N_4$	48.39	4.87	48.61	4.98	496
Xd	194—196	red prisms	73	$C_{40}H_{32}O_{11}N_4$	64.51	4.33	64.80	4.30	744
Xe	150—151	colorless prisms	80	$C_{17}H_{20}O_9N_4$	48.11	4.75	48.40	4.95	424
Xf	168—170	white amorphous powder	87	$C_{32}H_{26}O_9N_4$	62.95	4.97	63.10	4.30	610
Xg	163—166	colorless prisms	62	$C_{17}H_{20}O_9N_4$	48.11	4.75	48.38	4.99	424
Xh	175—176	colorless prisms	70	$C_{32}H_{26}O_9N_4$	62.95	4.29	62.86	4.60	610

Xa: 5-amino-7-methyl-3-(2,3,5-tri-*o*-acetyl)ribofuranosyloxazolo[4,5-*d*]pyrimidin-2-one
Xb: 5-amino-7-methyl-3-(2,3,5-tri-*o*-benzoyl)ribofuranosyloxazolo[4,5-*d*]pyrimidin-2-one
Xc: 5-amino-7-methyl-3-(2,3,4,6-tetra-*o*-acetyl)glucopyranosyloxazolo[4,5-*d*]pyrimidin-2-one
Xd: 5-amino-7-methyl-3-(2,3,4,6-tetra-*o*-benzoyl)glucopyranosyloxazolo[4,5-*d*]pyrimidin-2-one
Xe: 5-amino-7-methyl-3-(2,3,4-tri-*o*-acetyl)xylopyranosyloxazolo[4,5-*d*]pyrimidin-2-one
Xf: 5-amino-7-methyl-3-(2,3,4-tri-*o*-benzoyl)xylopyranosyloxazolo[4,5-*d*]pyrimidin-2-one
Xg: 5-amino-7-methyl-3-(2,3,4-tri-*o*-acetyl)arabopyranosyloxazolo[4,5-*d*]pyrimidin-2-one
Xh: 5-amino-7-methyl-3-(2,3,4-tri-*o*-benzoyl)arabopyranosyloxazolo[4,5-*d*]pyrimidin-2-one

Table III.  XIa—d

	mp (°C)	Appearance	Formura	Analysis (%) Calcd. C	Calcd. H	Found C	Found H	Mass (M^+)	Optical rotation $[\alpha]_D^{25}$
XIa	124—126	colorless prisms	$C_{11}H_{14}O_6N_4$	44.30	4.73	44.12	4.56	298	+ 2.0 (C, 1.0, H_2O)
XIb	150—151	colorless prisms	$C_{12}H_{16}O_7N_4$	43.90	4.91	43.73	4.99	328	+30.0 (C, 0.8, H_2O)
XIc	—	syrup	$C_{11}H_{14}O_6N_4$	44.30	4.73	44.03	4.97	298	+25.5 (C, 1.0, H_2O)
XId	—	syrup	$C_{11}H_{14}O_6N_4$	44.30	4.73	44.18	4.81	298	+85.5 (C, 1.0, H_2O)

XIa: 5-amino-7-methyl-3-D-ribofuranosyloxazolo[4,5-*d*]pyrimidin-2-one
XIb: 5-amino-7-methyl-3-D-glucopyranosyloxazolo[4,5-*d*]pyrimidin-2-one
XIc: 5-amino-7-methyl-3-D-xylopyranosyloxazolo[4,5-*d*]pyrimidin-2-one
XId: 5-amino-7-methyl-3-D-arabopyranosyloxazolo[4,5-*d*]pyrimidin-2-one

The deprotecting compounds (XIa—d) were prepared by treating Xa—h with ethanolic ammonia followed by purification with ion exchange chromatography using Amberlite IR-120B (H^+) and Amberlite IR-400 (OH^-). The properties of the compounds are summarized in Table III.

The nucleosides obtained (XIa—d) were shown homogeneous by thin-layer chromatography with $CHCl_3$: AcOEt: EtOH (8: 1: 1; v/v/v). The UV spectra of XIa—d exhibited the following absorptions: XIa, $\lambda_{max}^{0.1N\ NaOH}$ 301 mμ, $\lambda_{max}^{0.1N\ HCl}$ 300 mμ, λ_{max}^{EtOH}301 mμ, XIb, $\lambda_{max}^{0.1N\ NaOH}$ 302 mμ, $\lambda_{max}^{0.1N\ HCl}$ 300 mμ, λ_{max}^{EtOH} 301 mμ, XIc, $\lambda_{max}^{0.1N\ NaOH}$ 302 mμ, $\lambda_{max}^{0.1N\ HCl}$ 300 mμ, λ_{xeuu}^{EtOH} 301 mμ, XId, $\lambda_{max}^{0.1N\ NaOH}$ 302 mμ, $\lambda_{max}^{0.1N\ HCl}$ 300 mμ, λ_{max}^{EtOH} 303 mμ. These absorptions were in accord with those of N-3-alkyl derivatives of IX (R=CH_3, λ_{max}^{EtOH} 302 mμ, R=C_2H_5, λ_{max}^{EtOH} 303 mμ). The IR spectra exhibited absorption peaks characteristic of 5 membered lactone near 1735 cm^{-1}. Thus the position of sugar moiety was confirmed to be N-3 of oxazolo[4,5-*d*]pyrimidine (IX).

Also, the nuclear magnetic resonance (NMR) spectra of XIa—d revealed sharp doublets at δ 6.20 (J=4.0 cps), δ 6.12 (J=9.3 cps), δ 6.22 (J=6.5 cps) and δ 6.18 (J=7.2 cps) respectively, corresponding to C_1–H for sugar moiety. These spectral data favor that the compounds are β-anomer. In method 3), Ishido, *et al.*, have reported to give β-anomer by using *p*-toluenesulfonic acid or sulfamic acid as a catalyzer. The compounds obtained from method 3) were identified with the products (Xa—h) of method 1) and 2) by means of IR spectrum, melting point and mixed melting point determination.

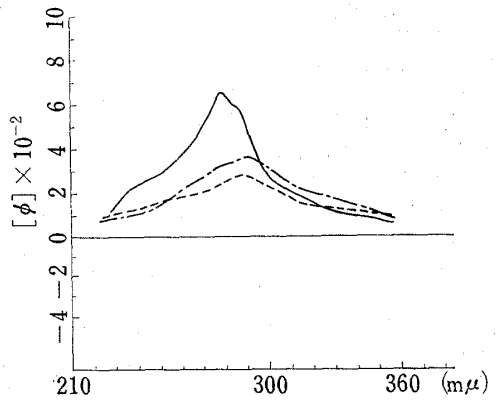


Fig. 1. ORD Curves

——: XIa
--------: XIb
——: 7-β-D-ribofuranosyltheophilline

The signs of the Cotton effect in XIa and XIb were both positive (Fig. 1). These results were well agreed with 7-β-D-ribofuranosyltheophilline.[9]

The above spectral data were similar with Xa—h, which were all consistent with the assigned structure.

Attempts to prepare glycosylpyrimidine (VIII) using ethoxycarbonyloxypyrimidine (VII)[1] and 1-bromo-2,3,5-tri-*O*-acetyl-D-ribofuranose were unsuccessful but gave IX.

The antitumor activities of II, VI, IX, Xc, Xd and Xg on *Yoshida sarcoma* were tested and found to be inactive.

Table IV. Resulting of Antitumor Examination

Drugs	Dose		
	10 mg/kg	2.5 mg/kg	1 mg/kg
II	13.5	—	21.6
VI	−6.7	—	−11.2
IX	−4.5	—	2.2
Xc	−2.5	−2.5	—
Xd	−12.5	25.5	—
Xg	0	0	—

Survival percentages on *Yoshida sarcoma* in Donryu rats saline-treated controls.

9) B. Heiferrich and R. Gootz, *Ber.*, **62**, 2788 (1929); Y. Ishido, H. Tanaka, T. Yoshino, M. Sekiya, K. Iwabuchi, and T. Sato, *Tetrahedron Letters*, **1967**, 5245.

Experimental

All melting points were measured on a Yanagimoto melting point apparatus and are uncorrected. IR spectra were taken on a Jasco infrared spectrophotometer IR-E and IR-S. UV spectra were measured on a Hitachi EPS-3T spectrophotometer. NMR spectra were run on a Jeol JNM-NH-60 at 60 Mcps using tetramethylsilane as an internal standard. Optical rotation were measured on a Yanagimoto direct reading polarimeter, model OR-10. ORD spectra were measured on a Jasco optical rotatory dispersion recorder, model ORD UV-5.

2,4-Diamino-6-methylpyrimidine (II)——To a solution of 1.84 g (0.08 atom) of sodium in 20 ml of absolute MeOH was added at 0—5° under stirring 1.91 g (0.02 mole) of guanidine hydrochloride and 1.66 g (0.02 mole) of acetoacetonitrile (III) was dropped into. The mixture was stirred for 30 min at room temperature and refluxed for 1.5 hr. After evaporation of the solvent *in vacuo*, H_2O was added to the residue and acidified with AcOH. The deposited crystals were collected and recrystallized from H_2O. mp 185°, colorless prisms, yields 1.85 g (75%). The identity of this product was verified by IR spectral comparison and mixed melting point determination with an authentic sample.

2-Amino-4-hydroxy-6-methyl-5-pyrimidinecarbonitrile (V)——To a solution of 0.23 g (0.01 atom) of sodium in 10 ml of absolute MeOH was added at 0—5° under stirring 0.48 g (0.005 mole) of guanidine hydrochloride and 0.92 g (0.005 mole) of IV was dropped into. The mixture was stirred for 30 min at room temperature and refluxed for 1 hr. After evaporation of the solvent *in vacuo*, H_2O was added to the residue and acidified with AcOH. The deposited crystals were collected and recrystallized from EtOH. mp>300°, colorless prisms, yields 0.51 g (68%). *Anal.* Calcd. for $C_6H_6ON_4$: C, 48.00; H, 4.03; N, 37.32. Found: C, 47.88; H, 3.80; N, 37.12. IR ν_{max}^{KBr} cm^{-1}: 2200 (nitrile). The product was identical with an authentic sample.

General Preparation of Xa—h——Method 1): To a solution of 0.046 g (0.002 atom) of sodium in 20 ml of absolute EtOH was dissolved 0.166 g (0.001 mole) of IX and 0.001 mole of halogenobenzoylsugar or halogenoacetylsugar was added. The mixture was refluxed for 10 hr on an oil bath at 100—110°. After evaporation of the solvent *in vacuo*, H_2O was added and unsoluble compounds were recrystallized from EtOH to give Xa—h.

Method 2): A mixture of 0.332 g (0.002 mole) of IX, 0.002 mole of halogenobenzoylsugar or halogenoacetylsugar, 0.5 g of $Hg(CN)_2$ and 200 ml of nitromethane was dried by an azeotropic distillation (finally 20 ml) and refluxed for 1 hr. After evaporation of the solvent, the residue was recrystallized from EtOH.

Method 3): A mixture of 0.332 g (0.002 mole) of IX and 0.002 mole of tetra-*O*-acetyl-*β*-D-sugar or 1-*O*-acetyl-tri-*O*-benzoyl-*β*-D-sugar was heated on an oil bath at 160—180° for 10 min, then 100 mg of *p*-toluenesufonic acid or 40 mg of sodium sulfamate was added. The mixture was heated on an oil bath at 100° for 30 min under a reduced pressure (25 mmHg) by a water aspirater. After cooled, the residue was recrystallized from EtOH with active charcoal.

General Preparation of XIa—d——To 50 ml of absolute EtOH saturated with NH_3 was dissolved 0.002 mole of Xa—h and allowed to stand for 5 days at 0—5°. On evaporation of the solvent *in vacuo* below 40°, the residual syrup was dissolved in 50% EtOH and treated with ion exchange resin Amberlite IR-400 and IR-120B successively. It was then crystallized from EtOH: AcOEt (1: 2) with active charcoal to give XIa—d.

Reaction of VII and 1-Bromo-2,3,5-tri-O-acetyl-D-ribofuranose——A mixture of 0.212 g (0.001 mole) of VII, 0.339 g (0.001 mole) of 1-bromo-2,3,5-tri-*O*-acetyl-D-ribofuranose and 10 ml of dioxane containing 0.5 ml of triethylamine was refluxed for 12 hr. After cooled, the deposited crystals were collected and recrystallized from dry DMF. mp>300°. This product was found to be IX from its IR spectrum.

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