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133. Takeo Naito, Katsujiro Ueno, and Fumiyoshi Ishikawa: Studies on Nucleosides and Nucleotides. VII.*1 Synthesis of 2-Amino-6-substituted-9-β-D-ribofuranosylpurines.

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In recent years a number of modified purine and pyrimidine derivatives have been synthesized for the investigation of the antagonists of nucleic acid. In the 2-amino-6-substituted-9-β-D-ribofuranosylpurines series, 6-hydroxy (guanosine),1) 6-amino,1) 6-thio (6-thioguanosine),2) 6-methylthio,3) and 6-chloro4) derivatives have been reported. This paper describes the preparation of several 2-amino-9-β-D-ribofuranosylpurine derivatives possessing nitrogen-containing groups at the 6-position, which might be expected to have antitumor activities.

6-Thioguanosine (I), synthesized by the method of Fox and coworkers,2) was used as the starting material for the present studies. Amination of the 6-thiol group of I with ammonia and amines was unsuccessful under the conditions investigated. This result is in contrast with the fact that the 4-thiol group of pyrimidine nucleosides (corresponding to the 6-thiol group of purines) is easily reacted with amines.5)

However, it was found that replacement of the 6-thiol group in I with amines could be achieved by way of the corresponding alkylthio derivatives. 6-Methylthio compound (IV) was prepared essentially according to the method reported by Robins and

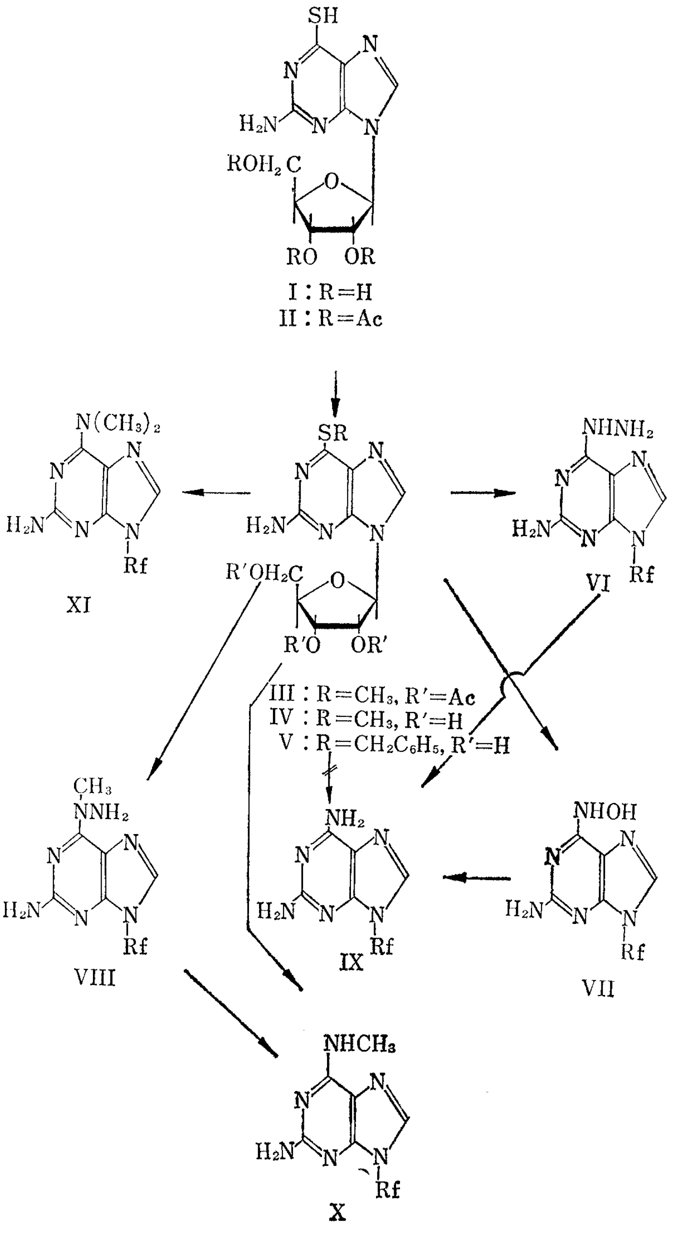

Chart 1. Rf : β-D-ribofuranosyl-

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Noell,[3] but the drawback of the reported method was that it was difficult to isolate the methylated product (Ⅳ) from inorganic salt formed. Tri-O-acetate (Ⅱ) of I was methylated in the same way to give tri-O-acetyl-methylthio compound (Ⅲ), but isolation of Ⅲ was also difficult. Then, in the case of treatment of I with benzyl chloride, the isolation of 6-benzylthio compound (V) was easily achieved, because V was separated as crystalline powder during reaction.

These methylthio and benzylthio derivatives (Ⅲ, Ⅳ, V) were found to be reacted easily with alkylamines, hydroxylamine and hydrazine derivatives. Thus, the compound (V), on heating with aqueous methylamine and dimethylamine in sealed tube afforded in good yields 2-amino-6-methylamino- (X) and 2-amino-6-dimethylamino-9-β-D-ribofuranosylpurine (Ⅺ), respectively. In similar way 2-amino-6-hydroxylamino-9-β-D-ribofuranosylpurine (Ⅶ) was obtained by condensation of V with hydroxylamine. Reaction of Ⅲ, Ⅳ and V with hydrazine and methylhydrazine proceeded faster than that with alkylamine or hydroxylamine, and 2-amino-6-hydrazino- (Ⅵ) and 2-amino-6-methylhydrazino-9-β-D-ribofuranosylpurine (Ⅷ) were given in excellent yields. When the methylhydrazine was condensed with benzylthio compound (V), only Ⅷ was obtained in good yields among two possible isomers, suggesting, as expected, that the methyl-substituted nitrogen in methylhydrazine is more nucleophilic. Structural elucidation of Ⅷ was based on the fact that reduction of Ⅷ gave 6-methylamino derivative (X).

Table I. Reactions of 2-Amino-6-alkylthio-9-β-D-ribofuranosylpurines with Various Amines

Material	Reagent	Reaction conditions (°C)	(hr.)	Products	m.p. (°C) (decomp.)	Yield (%)
Ⅳ	H_2NNH_2	100	3	Ⅵ		74.3
Ⅲ	〃	100	3	Ⅵ	218~221	77.0
V	〃	100	4	Ⅵ		88.2
V	H_2NOH	100	4	Ⅶ	224~225	55.1
V	$CH_3NH \cdot NH_2$	100	4	Ⅷ	216~218	75.7
V	CH_3NH_2	130~140	20	X	216~218 (picrate)	85.4
V	$(CH_3)_2NH$	130~140	20	Ⅺ	202~203	62.5

However, the direct reaction of these alkylated compounds (Ⅲ, Ⅳ, V) with ammonia failed to afford 2,6-diamino compound (Ⅸ).

Reduction of 6-hydrazino (Ⅵ) and 6-hydroxylamino (Ⅶ) derivatives with Raney nickel in boiling water afforded 2,6-diamino-9-β-D-ribofuranosylpurine (Ⅸ) in good yields, m.p. 240°, $[\alpha]_D$ $-39.6°$, UV $\lambda_{max}^{H_2O}$ mμ : 256, 280. The properties were in good agreement with those reported by Davoll and Lowy,[1] who prepared the compound (Ⅸ) only in low yield by condensation of chloromercuri salt of 2,6-dibenzamidopurine with triacetylribofuranosyl chloride. Reduction of 2-amino-6-methylhydrazino compound (Ⅷ) in the same manner as above furnished the 2-amino-6-methylamino derivative (X) which was identical with the product prepared by treatment of 6-benzylthio derivative (V) with methylamine.

Thus, the reaction described above provides a useful route for the preparation of 2,6-diamino-9-β-D-ribofuranosylpurine and its derivatives. The method may be generally applied to amination of groups which are impossible to be convert into amino group by direct amination.

The details of biological activities of compounds obtained will be reported elsewhere.

Experimental*3

2-Amino-6-mercapto-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)purine (II)——A well-stirred suspension

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*3 All melting points are uncorrected.

of 7.0 g. (23.4 mmoles) of 6-mercaptoguanosine (I) in 85 ml. of anhyd. pyridine was treated with 11.6 ml. (117 mmoles) of Ac_2O portionwise at room temperature. The solid was gradually dissolved and the resultant solution was kept at room temperature for 1 hr. The reaction mixture was concentrated to dryness *in vacuo*. The residue was crystallized from MeOH to give 7.2 g. (72.3%) of crystals, m.p. 235∼236° (decomp.). Further recrystallization from MeOH afforded taper prisms, m.p. 241∼242° (decomp.). *Anal.* Calcd. for $C_{16}H_{19}O_7N_5S$: C, 45.17; H, 4.50; N, 16.46. Found: C, 45.31; H, 4.68; N, 16.46.

2-Amino-6-methylthio-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)purine (III)——A solution of 1.17 g. (2.75 mmoles) of II in 13.8 ml. (5.5 mmoles) of 0.4*N* NaOH was stirred vigorously at room temperature for 10 min. while 0.27 ml. of Me_2SO_4 was added in portions. A separated oil was taken up into $CHCl_3$, washed with H_2O and dried over Na_2SO_4. After removal of the solvent *in vacuo*, the residual sirup was triturated with petr. ether to give a glassy solid. This material was dissolved in AcOEt and passed through a column of Al_2O_3 (20 g.). Evaporation of the solvent gave amorphous hygroscopic powder, which resisted attempts at crystallization. *Anal.* Calcd. for $C_{17}H_{24}O_7N_5S$: C, 46.46; H, 4.82; N, 15.94. Found: C, 46.74; H, 4.69; N, 15.58.

2-Amino-6-benzylthio-9-β-D-ribofuranosylpurine (V)——A mixture of 3.08 g. (10 mmoles) of I and 1.38 ml. (12 mmoles) of benzyl chloride in 40 ml. of 0.5*N* NaOH was vigorously stirred at room temperature for 1.5 hr. during which time white solid separated. The solid was collected, washed with H_2O and dried *in vacuo* below 50°. Yield was 3.9 g. (79.5%), m.p. 80∼85°. *Anal.* Calcd. for $C_{17}H_{19}O_4N_5S$: C, 52.43; H, 4.94; N, 18.00. Found: C, 52.71; H, 5.25; N, 17.78.

2-Amino-6-hydrazino-9-β-D-ribofuranosylpurine (VI)——i) A solution of 4.9 g. (12.6 mmoles) of V in 5 ml. of 40% NH_2NH_2 was heated on boiling water bath for 4 hr. The reaction mixture was concentrated to a half volume *in vacuo*. After addition of 5 ml. of EtOH, it was concentrated again until the crystals began to separate. The mixture was kept in refrigerator overnight and the crystals were collected, m.p. 212∼214° (decomp.). This product was recrystallized from H_2O to colorless prisms, 3.30 g. (88.2%), m.p. 218∼221° (decomp.), $[\alpha]_D^{21}$ −46.2° (c=0.93, 0.1*N* HCl). UV $\lambda_{max}^{H_2O}$ mμ (ε): 259 (9770), 283 (12700). *Anal.* Calcd. for $C_{10}H_{15}O_4N_7 \cdot 1/2H_2O$: C, 39.21; H, 5.27; N, 32.01. Found: C, 39.39; H, 5.13; N, 32.10.

ii) A mixture of 1.54 g. (5 mmoles) of I and 0.57 ml. (6 mmoles) of Me_2SO_4 in 20 ml. of 0.5*N* NaOH was shaken vigorously at room temperature for 30 min. After neutralization with AcOH the mixture was concentrated to dryness *in vacuo*. Ten milliliters of 40% NH_2NH_2 was added to residual sirup. The mixture was heated on boiling water bath for 3 hr. and concentrated *in vacuo* to remove the excess NH_2NH_2 by co-distillation with H_2O. Refrigerating overnight, the solid was filtered, washed with H_2O and dried, 1.14 g. (74.3% from I), m.p. 213∼215° (decomp.). This material was identical with that obtained above.

iii) A mixture of 2.6 g. (6.1 mmoles) of II and 0.61 g. (6.2 mmoles) of Me_2SO_4 in 60 ml. of 0.4*N* NaOH was vigorously shaken at room temperature for 30 min. After the mixture acidified with AcOH was concentrated to dryness *in vacuo* and 10 ml. of 40% NH_2NH_2 was added to residue. The mixture was heated on boiling water bath for 3 hr. Work-up in the same manner as described above afforded VI as crystals of m.p. 215∼217° (decomp.), 1.4 g. (77.0% from II), identical with that obtained above.

2-Amino-6-methylhydrazino-9-β-D-ribofuranosylpurine (VIII)——A solution of 1.94 g. (5 mmoles) of V in 10 ml. of 40% $MeNH \cdot NH_2$ was heated on boiling water bath for 4 hr. The reaction mixture was worked up in the same manner as described for VI. The product was recrystallized from MeOH-H_2O to colorless needles, 1.18 g. (75.7%), m.p. 216∼218° (decomp.), $[\alpha]_D^{21}$ −36.9° (c=0.77, 0.1*N* HCl). UV $\lambda_{max}^{H_2O}$ mμ (ε): 263 (9750), 288 (14500). *Anal.* Calcd. for $C_{11}H_{17}O_4N_7$: C, 42.44; H, 5.33; N, 31.50. Found: C, 42.86; H, 5.33; N, 30.79.

2-Amino-6-hydroxylamino-9-β-D-ribofuranosylpurine (VII)——A solution of H_2NOH in MeOH was prepared by neutralization of 7.0 g. of $H_2NOH \cdot HCl$ with MeONa and filtration of precipitated NaCl. To this solution was added 0.50 g. (1.28 mmoles) of V and the mixture was heated at 100° for 4 hr. in a sealed tube. The yellow solution was evaporated to dryness and the crystalline residue was triturated with Et_2O and filtered. The product was recrystallized from MeOH-H_2O to colorless tiny prisms, 0.21 g. (55.1%), m.p. 224∼225° (decomp.), $[\alpha]_D^{21}$ −24.3° (c=0.95, 0.1*N* HCl). UV $\lambda_{max}^{H_2O}$ mμ (ε): 264 (shoulder) (9400), 282 (11900). *Anal.* Calcd. for $C_{10}H_{14}O_5N_6 \cdot H_2O$: C, 37.97; H, 5.10; N, 26.57. Found: C, 37.97; H, 5.02; N, 26.72.

2,6-Diamino-9-β-D-ribofuranosylpurine (IX)——i) A mixture of 2.0 g. (6.5 mmoles) of VI and 10 ml. of W-7 Raney Ni in 80 ml. of H_2O was refluxed for 1 hr. The mixture was filtered, the catalysts leached several times with boiling H_2O. The filtrate and washings were combined and evaporated *in vacuo* to a sirup which was triturated with EtOH to crystalline solid. The product was recrystallized from H_2O to colorless prisms, 1.30 g. (70.7%), m.p. 238∼240°, $[\alpha]_D^{21}$ −39.6° (c=0.66, 0.1*N* HCl). UV $\lambda_{max}^{H_2O}$ mμ (ε): 256 (10700), 280 (11100). *Anal.* Calcd. for $C_{10}H_{14}O_4N_6$: C, 42.55; H, 5.00; N, 29.78. Found: C, 42.82; H, 4.64; N, 29.93.

ii) A mixture of 0.50 g. (1.88 mmoles) of VII and 3 ml. of W-7 Raney Ni in 30 ml. of H_2O was refluxed for 1 hr. The work-up in the same manner as above afforded colorless prisms, 0.23 g. (56.2%), m.p. 235∼237°, identical with a sample obtained above.

2-Amino-6-methylamino-9-β-D-ribofuranosylpurine (X)——i) A solution of 1.0 g. (2.57 mmoles) of V in 7 ml. of 40% $MeNH_2$ was heated in a sealed tube at 130~140° for 20 hr. After cooling, benzylmercaptane was removed by extracting with Et_2O and aqueous layer was concentrated to dryness *in vacuo.* The residual sirup was triturated with Et_2O and yellow amorphous solid, 0.65 g. (86.4%), m.p. 80~90°, $[\alpha]_D^{21}$ −38.8° (c=1.02, 0.1*N* HCl). UV $\lambda_{max}^{H_2O}$ mμ (ε): 265 (shoulder) (10300), 280 (13100).

Attempts to crystallize this solid from a variety of solvents failed. The picrate of this compound showed m.p. 215~218° (decomp.). *Anal.* Calcd. for $C_{17}H_{19}O_{11}N_9$: C, 38.83; H, 3.64; N, 24.00. Found: C, 38.79; H, 3.59; N, 24.34.

ii) A mixture of 0.50 g. (1.6 mmoles) of Ⅷ and 3 ml. of W-7 Raney Ni in 30 ml. of H_2O was refluxed for 1 hr. The mixture was filtered, the catalysts were washed several times with hot H_2O. The filtrate and washings were combined and evaporated to dryness *in vacuo.* The residual sirup was triturated with Et_2O and amorphous solid separated, 0.30 g. (63.3%), UV $\lambda_{max}^{H_2O}$ mμ: 265 (shoulder), 280.

The picrate showed m.p. 216~218° (decomp.). *Anal.* Calcd. for $C_{17}H_{19}O_{11}N_9$: N, 24.00. Found: N, 24.35. A mixed melting point with a sample prepared above showed no depression.

2-Amino-6-dimethylamino-9-β-D-ribofuranosylpurine (XI)——A solution of 1.0 g. (2.57 mmoles) of V in 7 ml. of 40% Me_2NH was heated in a sealed tube at 130~140° for 20 hr. After cooling, benzylmercaptane was removed by extracting with Et_2O and aqueous layer was evaporated to dryness *in vacuo.* A pale yellow amorphous residue was recrystallized from Me_2CO-MeOH to white tiny prisms, 0.5 g. (62.5%), m.p. 202~203° (decomp.), $[\alpha]_D^{21}$ −44.2° (c=1.47, 0.1*N* HCl). UV $\lambda_{max}^{H_2O}$ mμ (ε): 228 (19100), 268 (shoulder) (11500), 284 (15600). *Anal.* Calcd. for $C_{12}H_{18}O_4N_6$: C, 46.44; H, 5.81; N, 27.09. Found: C, 45.85; H, 6.02; N, 27.35.

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Summary

In the 2-amino-6-substituted-9-β-D-ribofuranosylpurines series, 6-hydrazino (Ⅵ), 6-methylhydrazino (Ⅷ), 6-hydroxylamino (Ⅶ), 6-methylamino (X), and 6-dimethylamino (Ⅺ) derivatives were synthesized by condensation of 6-alkylthioguanosines with respective amines. Ⅵ and Ⅶ were reduced with Raney nickel to 2,6-diamino derivative (Ⅸ). Similarly, Ⅷ was also reduced with Raney nickel to X.

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134. Yoshio Sakurai[*1] and Mahmoud M. El-Merzabani[*2]: Carcinostatic Methanesulfonic Acid Esters of Some Aminoglycols.

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Numerous reports[1~4] have been published on the antitumor activity of sulfonic acid esters of glycols and aminoglycols, out of which dimethanesulfonic ester of 1,4-butanediol (Myleran) had been used at present as a clinical antileukemic agent of practical value.

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