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Studies of Nucleosides and Nucleotides. LXXX.[1] Purine Cyclonucleosides. (38). Synthesis of 6-Substituted Purine 2′-Azido- and 2′-Amino-2′-deoxyribofuranosides

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Starting from 2′-azido-2′-deoxyadenosine (**1**), sugar-acetylated 6-chloro compound (**5**) was synthesized by successive deamination, acetylation and chlorination. Using the compound **5** as intermediate 6-monomethylamino-, dimethylamino-, mercapto- and methylthio 2′-azido nucleosides (**6, 8, 11** and **12**) were obtained. Palladium-catalyzed hydrogenation of compound **6** and **8** afforded 2′-amino nucleosides (**7** and **9**) respectively.

Keywords——nitrite deamination; Vilsmeier reagent; UV; IR; paper chromatography; TLC

Recently, we have developed[3,4] a method for synthesizing 2′-azido-2′-deoxyadenosine (**1**) starting from the naturally occurring adenosine *via* 8,2′-O-cyclonucleoside[5] as an intermediate. Since this method is suitable for the large scale synthesis, we utilize the compound **1** as a starting material for the synthesis of various purine nucleosides having Cl, SH, SMe, NHMe, and $N(Me)_2$ groups at 6-position. Ribonucleosides having these substituents at 6-position of purine residues have been proved to be active as antimetabolites.[6] Recent finding[7] that an antibiotic 2′-deoxy-2′-aminoguanosine also have antibacterial and anticancer activities prompted us to investigate the synthesis of compounds mentioned above.

2′-Azido-2′-deoxyadenosine (**1**) was deaminated first with sodium nitrite in acetic acid to obtain 6-oxy compound, 2′-azido-2′-deoxy-inosine (**2**). The yield was variable between 60—77% in several runs. The compound **2** was characterized by comparison with an authentic sample synthesized from 8,2′-O-cyclonucleoside by the attack of azide anion followed by the elimination of 8-oxy group.[8,9] Palladium catalyzed hydrogenolysis of the compound **2** gave 2′-amino-2′-deoxyinosine (**3**) as a form of syrup. Presence of the amino group was indicated by a positive ninhydrin test. In order to obtain crystalline compound, the compound **3** was derivatized to a hydrochloride which was obtained in a yield of 89% calculated from the compound **2**. Ultraviolet (UV) absorption properties and elemental analytical value supported the structure to be correct.

2′-Azido-2′-deoxyinosine (**2**) was then protected with acetyl groups at 3′- and 5′-hydroxyls by the treatment with acetic anhydride in pyridine. Crystalline 3′,5′-di-O-acetyl-2′-azido-2′-deoxyinosine (**4**) thus obtained, was subjected to chlorination using thionyl chloride and DMF[10] as described previously for the chlorination of inosine derivatives.[11,12] 6-Chloro-

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6) See for instance, P. Langer, "Antimetabolites of Nucleic Acid Metabolism," Gordon and Breach, New York, 1974.
7) T. Nakanishi, F. Tomita, and T. Suzuki, *Agr. Biol. Chem.*, **38**, 2465 (1974).
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10) A. Vilsmeier and E. Haack, *Ber.*, **60B**, 119 (1927).
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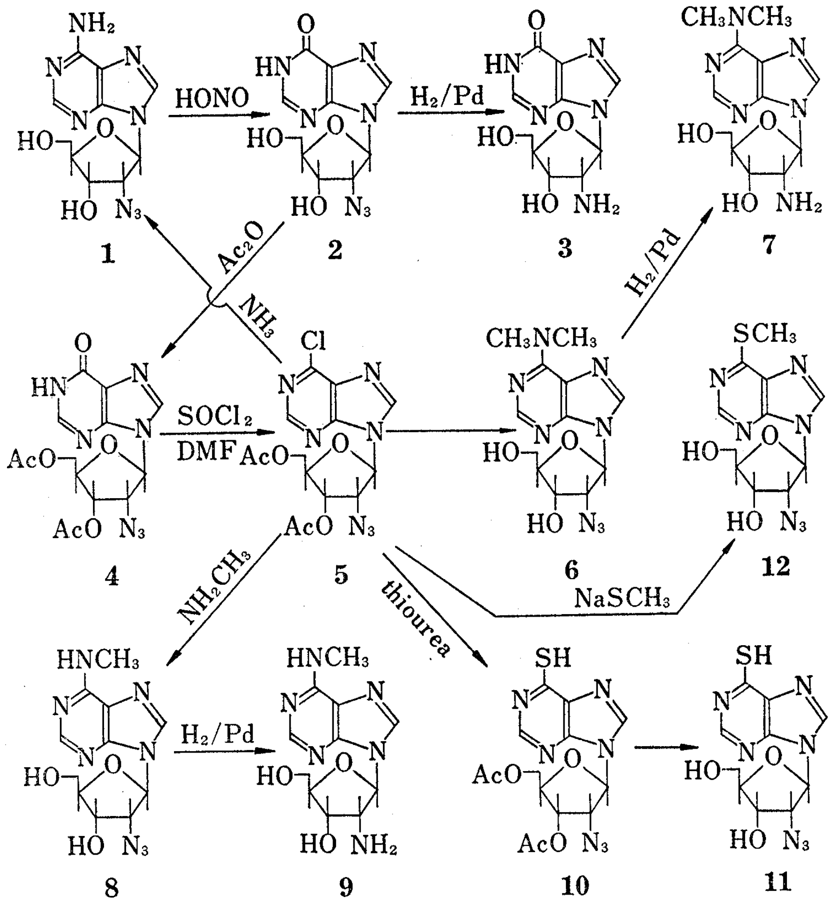

9-(3′,5′-di-O-acetyl-2′-azido-2′-deoxy-β-D-ribofuranosyl)purine (**5**) was isolated and characterized by its UV absorption spectra, which were closely resembled to those of 6-chloropurine riboside.[13] The structure was further supported by the fact that the compound (**5**) gave 2′-azido-2′-deoxyadenosine (**1**) by the treatment with methanolic ammonia at 100° for 4 hr.

The 6-chloro compound **5** was then allowed to react with aqueous dimethylamine at 100° for 2 hr. N^6-Dimethyl-2′-azido-2′-deoxyadenosine (**6**) was obtained as a glass showing UV absorption similar to that of N^6-dimethyl-adenosine[14] and infrared (IR) absorption band at 2100 cm^{-1}, which was assigned to the azido group. When the compound (**6**) was hydrogenolyzed with palladium charcoal as catalyst, N^6-dimethyl-2′-amino-2′-deoxyadenosine (**7**) was obtained again as a hard syrup. Derivatization to a hydrochloride failed to give a crystalline compound, but its UV absorption having $\lambda_{max}^{H_2O}$ at 274.5 nm and positive ninhydrin spray test suggested the correct structure for the compound **7**. Comparison of this sample with a sample synthesized *via* another route[9] showed the same *Rf* values in paper chromatography in two solvent system. The chloro compound **5** was next allowed to react with aqueous monomethylamine at 100° for 3 hr. N^6-Methyl-2′-azido-2′-deoxyadenosine (**8**) obtained as a syrup was hydrogenated over palladium catalyst to give N^6-methyl-2′-amino-2′-deoxyadenosine (**9**), which was obtained as dihydrochloride of mp 202—204° in a yield of 52%. Elemental analysis and UV absorption properties resembled to those of N^6-methyladenosine[12] and positive ninhydrin spray test confirmed the structure.

13) G.B. Brown and V.S. Weliky, *J. Biol. Chem.*, **204**, 1019 (1953).
14) J.M. Andrews and W.E. Barker, *J. Chem. Soc.*, **1958**, 2768.

In order to obtain analogs of 6-thioinosine, the compound **5** was treated with thiourea in refluxing *n*-propyl alcohol. Three hrs' reaction gave 6-mercapto-9-(3′,5′-di-O-acetyl-2′-azido-2′-deoxy-β-D-ribofuranosyl)purine (**10**) in a yield of 46%. Elemental analysis gave the correct value and UV absorption properties resembling those of 6-mercaptopurine riboside[15] proved the structure of **10**. For deprotection the compound **10** was treated with methanolic ammonia to give 6-mercapto-9-(2′-azido-2′-deoxy-β-D-ribofuranosyl)purine (**11**) in a yield of 65%. Elemental analysis and UV absorption showed the structure be correct.

The compound **5** was finally allowed to react with sodium methylmercaptide in dioxane-water mixture at room temperature for 12 hr. 6-Methylthio-(2′-azido-2′-deoxy-D-ribofuransyl)purine (**12**) was obtained in a yield of 58%. The structure of the compound **12** was supported by its IR absorption band at 2110 cm^{-1} and UV absorption spectra resembled to those of 6-methylthiopurine riboside.[15] Biological properties of the compounds synthesized as above will be reported in subsequent papers.

Experimental[16]

2′-Azido-2′-deoxyinosine (2)——2′-Azido-2′-deoxyadenosine (1) (94.5 mg, 0.32 mmol) was dissolved in 80% AcOH (10 ml) and $NaNO_2$ (235 mg, 10 equiv) was added. The mixture was kept at 37° for 12 hr. After checking the reaction extent by TLC ($CHCl_3$–EtOH, 5: 1), the solvent was evaporated *in vacuo*. After trace of AcOH was totally removed by coevaporation with H_2O, the residue was dissolved in pyridine and evaporated *in vacuo*. Recrystallization of the residue from EtOH gave 2 as amorphous powder in a yield of 77%. UV $\lambda_{max}^{H_2O}$ 248 nm, $\lambda_{max}^{pH\,2}$ 248, $\lambda_{max}^{pH\,12}$ 254. PPC: *Rf*(A) 0.35, *Rf*(B) 0.64.

2′-Amino-2′-deoxyinosine (3)——2′-Azido-2′-deoxyinosine (2) (146 mg, 0.5 mmol) was dissolved in H_2O (23 ml) and AcOH (5 ml). To the solution H_2-gas was absorbed for the 1 hr with stirring in the presence of 10% Pd-charcoal (50 mg). The catalyst was removed by filtration and washed with H_2O. The filtrate and washings were combined and evaporated *in vacuo*. The residual syrup was dissolved in H_2O (1 ml) and 1 N HCl (1 ml) was added. Evaporation and recrystallization of the residue from EtOH gave 3, mp 180—185°, in a yield of 185 mg (87%). *Anal.* Calcd. for $C_{10}H_{13}N_5O_4 \cdot HCl \cdot 1/2H_2O$: C, 38.40; H, 4.84; N, 22.40; Cl, 11.34. Found: C, 38.15; H, 4.74; N, 22.05; Cl, 12.20. UV: $\lambda_{max}^{H_2O}$ 248.5 nm (ε 11100) $\lambda_{max}^{0.1N\,HCl}$ 249 (11700) $\lambda_{max}^{0.1N\,NaOH}$ 254 (12300). PPC: *Rf*(A) 0.05, *Rf*(B) 0.67, *Rf*(C) 0.17.

3′,5′-Di-O-acetyl-2′-azido-2′-deoxyinosine (4)——2′-Azido-2′-deoxyinosine (2) (146 mg, 0.5 mmol) was dissolved in pyridine (2 ml) and Ac_2O (1 ml) was added to the solution. The reaction mixture was kept at room temperature for 1 hr. The solvent was evaporated *in vacuo* and trace of AcOH was removed by evaporation with H_2O several times. The residue was recrystallized from EtOH to give **4**, mp 180—181°, in a yield of 110 mg (59%). *Anal.* Calcd. for $C_{14}H_{15}N_7O_6$: C, 44.56; H, 4.01; N, 25.99. Found: C, 44.56; H, 3.82; N, 25.81. UV: $\lambda_{max}^{50\%\,EtOH}$ 248 nm. IR: ν_{max}^{KBr} 2125 cm^{-1} (N3). The sample was identical with that synthesized previously[8] by criteria of *Rf* 0.42 in TLC ($CHCl_3$–EtOH, 7: 1).

6-Chloro-9-(3′,5′-di-O-acetyl-2′-azido-2′-deoxy-β-D-ribofuranosyl)purine (5)——Thionyl chloride (0.2 ml) was dissolved in anhydrous $CHCl_3$ (6 ml). Anhydrous DMF (0.1 ml) was added to the $CHCl_3$ solution and the mixture was kept at room temperature for 10 min. To the mixture 3′,5′-di-O-acetyl-2′-azido-2′-deoxyinosine (4) (95 mg, 0.25 mmol) was added. The reaction mixture was heated at refluxing temperature for 3 hr under exclusion of moisture. After checking the reaction extent by TLC, $CHCl_3$ was evaporated *in vacuo*. The residue was poured into ice-water (30 ml) with stirring. The nucleoside was extracted with $CHCl_3$ (30 ml), washed with $NaHCO_3$ aq. and H_2O, and dried over $MgSO_4$. Evaporation of the solvent gave **5** as a hard syrup. UV: $\lambda_{max}^{50\%\,EtOH}$ 251 (shoulder) 257, 262.5 nm; $\lambda_{max}^{pH\,2}$ 250 (sh), 256, 262; $\lambda_{max}^{pH\,12}$ 251 (sh), 257, 262.5 TLC ($CHCl_3$–EtOH, 15: 1): *Rf* 0.60.

2′-Azido-2′-deoxyadenosine (1)——3′,5′-Di-O-acetyl-2′-azido-6-chloro compound (5) (obtained from 0.25 mmol of 4) was sealed in a steel tube with methanolic ammonia (saturated at 0°, 5 ml). The tube was heated at 100° for 4 hr. The reaction mixture was evaporated *in vacuo* to a syrupy residue, which was crystallized from H_2O to give 2′-azido-2′-deoxyadenosine, mp 208—210°, in a yield of 39.2 mg (54%). This sample was identical with an authentic 2′-azido-2′-deoxyadenosine[3] by criteria of mixed mp test, UV absorp-

15) J.J. Fox, I. Wempen, A. Hampton, and I.L. Doerr, *J. Am. Chem. Soc.*, **80**, 1969 (1958).

16) UV absorption spectra were taken with Hitachi EPS-3T and 124 spectrophotometer. IR spectra were taken with a Hitachi EPL-L spectrophotometer. NMR spectra were taken with a Hitachi R-22 spectrometer operated at 90 MHz using tetramethylsilane as internal standard. Paper chromatography was performed on Toyo Roshi filter paper No. 51A in solvent systems: A, *n*-BuOH–H_2O (84: 16); B, isoPrOH–conc. NH_4OH–H_2O, (7: 1: 2); C, *n*-BuOH–AcOH–H_2O (5: 2: 3). TLC was performed in Kieselgel HF-254 plates.

tion properties ($\lambda_{max}^{H_2O}$ 257 nm, $\lambda_{max}^{pH\,2}$ 257, $\lambda_{max}^{pH\,12}$ 259) and *Rf's* in paper chromatography: *Rf*(A) 0.20, *Rf* (B) 0.40.

N^6-Dimethyl-2′-azido-2′-deoxyadenosine (6)——The compound **5** (obtained from 0.6 mmol of **4**) was sealed in a steel tube with 40% aqueous dimethylamine (20 ml) and heated at 100° for 2 hr. The solvent was evaporated *in vacuo* and a syrupy residue was obtained in a yield of *ca.* 60% estimated by optical density. UV: $\lambda_{max}^{50\%EtOH}$ 275 nm, $\lambda_{max}^{pH\,2}$ 268.5; $\lambda_{max}^{pH\,12}$ 273.5. IR: $\nu_{max}^{CHCl_3}$ 2100 cm^{-1} (N_3). TLC ($CHCl_3$–EtOH, 7: 1): *Rf* 0.59.

N^6-Dimethyl-2′-amino-2′-deoxyadenosine (7)——N^6-Dimethyl-2′-azido compound (**6**) (obtained as above from 0.6 mmol of **4**) was dissolved in a mixture of H_2O (23 ml) and AcOH (5 ml). Palladium charcoal (20%, 80 mg) was added to the solution and H_2-gas was absorbed with stirring for 2 hr. The catalyst was filtered off, washed with H_2O, the filtrate and washings were combined and evaporated *in vacuo*. A glass was obtained in a yield of 240 mg (*ca.* 50%). UV: $\lambda_{max}^{H_2O}$ 274.5 nm, $\lambda_{max}^{pH\,2}$ 266.5, $\lambda_{max}^{pH\,12}$ 274.5. PPC: *Rf*(B) 0.71, *Rf*(C) 0.66. TLC ($CHCl_3$–EtOH, 3: 1) *Rf* 0.18. These values were similar to those reported previously.[8]

N^6-Methyl-2′-azido-2′-deoxyadenosine (8)——Diacetyl-6-chloro compound (**5**) (prepared from 0.6 mmol of **4**) was sealed in a steel tube with 30% monomethylamine aq (20 ml). The tube was heated at 100° for 3 hr. The solvent was evaporated *in vacuo* to give a syrup. Yield was 48% estimated by optical density. UV: $\lambda_{max}^{50\%EtOH}$ 266.5 nm, $\lambda_{max}^{pH\,2}$ 263, $\lambda_{max}^{pH\,12}$ 266. IR: $\nu_{max}^{CHCl_3}$ 2110 cm^{-1} (N_3). TLC ($CHCl_3$–EtOH, 5: 1): *Rf* 0.59.

N^6-Methyl-2′-amino-2′-deoxyadenosine (9) (Hydrochloride)——The compound **8** (obtained as above from 0.6 mmol of **4**) was dissolved in a mixture of H_2O (23 ml) and AcOH (5 ml). To the solution 20% Pd-C (50 mg) was added and H_2-gas was absorbed for 1 hr with stirring. The catalyst was removed by filtration and washed with hot H_2O. The filtrate and washings were combined and evaporated to a residue. The residue was taken up in a small amount of H_2O and 1 N HCl (2 ml) was added. The solution was evaporated and the residue was crystallized from EtOH. The compound **9** (HCl salt), mp 202—204°, was obtained in a yield of 110 mg (52%). *Anal.* Calcd. for $C_{11}H_{16}N_6O_3 \cdot 2HCl \cdot H_2O$: C, 35.59; H, 5.43; N, 22.64; Cl, 19.10. Found: C, 35.16; H, 4.96; N, 22.55, Cl, 19.95. UV $\lambda_{max}^{H_2O}$ 266 (ε 16300), $\lambda_{max}^{pH\,2}$ 262 (18000), $\lambda_{max}^{pH\,12}$ 266 (16400). PPC: *Rf*(A) 0.18, *Rf*(B) 0.84, *Rf*(C) 0.43. TLC ($CHCl_3$–EtOH, 3: 1): *Rf* 0.14.

6-Mercapto-9-(3′,5′-di-O-acetyl-2′-azido-2′-deoxy-β-D-ribofuranosyl)purine (10)——The compound **5** (obtained from 0.5 mmol of **4**) was dissolved in *n*-PrOH (10 ml) and thiourea (190 mg, 5 equiv) was added. The reaction mixture was heated at refluxing temperature for 30 min. The solvent was evaporated *in vacuo* and the residue was extracted with $CHCl_3$. The $CHCl_3$ solution was dried over $MgSO_4$ and evaporated to give a residue, which was recrystallized from AcOEt. The compound 10, mp 150—155°, was obtained in a yield of 100 mg (46%). *Anal.* Calcd. for $C_{14}H_{15}N_7O_5S$: C, 42.74; H, 3.84; N, 24.93, S, 8.15. Found: C, 42.46; H, 3.68; N, 24.74; S, 8.15. UV: $\lambda_{max}^{50\%EtOH}$ 323.5 nm, λ_{max}^{pH2} 323, λ_{max}^{pH12} 315. IR: $\nu_{max}^{CHCl_3}$ 2110 cm^{-1}. TLC ($CHCl_3$–EtOH, 15: 1): *Rf* 0.45.

6-Mercapto-9-β-(2′-azido-2′-deoxy-D-ribofuranosyl)purine (11)——The compound **10** (80 mg) was dissolved in methanolic ammonia (saturated at 0°, 10 ml) and kept at room temperature for 12 hr. The solvent was removed *in vacuo* and the residue was recrystallized from H_2O containing a small amount of $Na_2S_2O_3$.[17] The sample colorized at 190° and melted at 212—214°. *Anal.* Calcd. for $C_{10}H_{11}N_7O_3S \cdot 1/2H_2O$: C, 37.73; H, 3.80; N, 30.80; S, 10.07. Found: C, 37.53; H, 3.33; N, 30.78; S, 10.18. UV $\lambda_{max}^{50\%EtOH}$ 323.5 nm (ε 23000), $\lambda_{max}^{pH\,2}$ 324 nm (23000), $\lambda_{max}^{pH\,12}$ 315 nm (22800). IR: ν_{max}^{KBr} 2110 cm^{-1} (N_3). PPC: *Rf*(A) 0.92, *Rf*(B) 0.84, *Rf*(C) 0.89. TLC ($CHCl_3$–EtOH, 10: 1): *Rf* 0.10.

6-Methylthio-9-β-(2′-azido-2′-deoxy-D-ribofuranosyl)purine (12)——The compound **5** (obtained from 0.5 mmol of **4**) was dissolved in dioxane (20 ml) and 20% $NaSCH_3$ aq. (1.5 ml) was added. The reaction mixture was kept at room temperature for 12 hr. After the H_2O-layer was removed by suction with a pipett, the dioxane-layer was neutralized with 1 N HCl (5 ml). The solvent was evaporated *in vacuo*, the residue coevaporated with H_2O, and extracted with a mixture of *n*-BuOH (30 ml) and H_2O (30 ml). From the BuOH solution the compound **12** was obtained as a hard syrup in a yield of 58%. UV: $\lambda_{max}^{50\%EtOH}$ 287, 292 nm, $\lambda_{max}^{pH\,2}$ 287, 292; $\lambda_{max}^{pH\,12}$ 287, 292. IR: $\nu_{max}^{CHCl_3}$ 2110 cm^{-1} (N_3). PPC: *Rf*(A) 0.93, *Rf*(B) 0.94, *Rf*(C) 0.92. TLC ($CHCl_3$–EtOH, 15: 1): *Rf* 0.35.

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17) M. Ikehara and M. Muraoka, *Chem. Pharm. Bull.* (Tokyo), **20**, 550 (1972).