Studies of Nucleosides and Nucleotides. LXXX.\textsuperscript{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\textsuperscript{3,4} a method for synthesizing 2'-azido-2'-deoxyadenosine (1) starting from the naturally occurring adenosine via 8,2'-O-cyclonucleoside\textsuperscript{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, SM, 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.\textsuperscript{6} Recent finding\textsuperscript{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.\textsuperscript{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\textsuperscript{10} as described previously for the chlorination of inosine derivatives.\textsuperscript{11,12} 6-Chloro-

\textsuperscript{2} Location: 133-1 Yamaidakami, Suita, 555, Japan.
\textsuperscript{4} M. Ikehara, T. Maruyama, and H. Miki, submitted for publication.
\textsuperscript{5} M. Ikehara, Accounts Chem. Res., 2, 47 (1969), and subsequent papers.
\textsuperscript{10} A. Vilsmeier and E. Haack, Ber., 60B, 119 (1927).
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.\(^\text{13}\) 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\(^\text{14}\) 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 \(\lambda_{max}^{\text{UV}}\) 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\(^\text{9}\) showed the same \(R_f\) 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\(^8\)-Methyl-2'-azido-2'-deoxyadenosine (8) obtained as a syrup was hydrogenated over palladium catalyst to give N\(^8\)-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\(^8\)-methyladenosine\(^\text{12}\) and positive ninhydrin spray test confirmed the structure.


In order to obtain analogs of 6-thioguanine, the compound 5 was treated with thiourea in refluxing n-propyl alcohol. Three hrs' reaction gave 6-mercaptopurine (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-mercaptopurine (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-β-d-ribofuranosyl)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.\(^{16}\) 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 NaN\(_3\) (235 mg, 10 equiv) was added. The mixture was kept at 37°C for 12 hr. After checking the reaction extent by TLC (CH\(_2\)Cl\(_2\)-EtOH, 5:1), the solvent was evaporated in vacuo. After trace of AcOH was totally removed by coevaporation with H\(_2\)O, 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 \(\lambda_{max}^{nm} 248, 248, 248, 254\). PPC: R\(_f\) (A) 0.35, R\(_f\) (B) 0.64.

2'-Amino-2'-deoxyinosine (3)—2'-Azido-2'-deoxyinosine (2) (146 mg, 0.5 mmol) was dissolved in H\(_2\)O (23 ml) and AcOH (5 ml). To the solution H\(_2\)gas was absorbed for the hr with stirring in the presence of 10% Pd-charcoal (50 mg). The catalyst was removed by filtration and washed with H\(_2\)O. The filtrate and washings were combined and evaporated in vacuo. The residual syrup was dissolved in H\(_2\)O (1 ml) and 1 N HCl (1 ml) was added. Evaporation and recrystallization of the residue from EtOH gave 3, mp 180—185°C, in a yield of 185 mg (87%). Anal. Calcd. for C\(_6\)H\(_{15}\)N\(_4\)O\(_6\): H\(_2\)O: 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: \(\lambda_{max}^{nm} 248.5\) nm (e 11100) \(\lambda_{max}^{nm} 249\) (11700) \(\lambda_{max}^{nm} 254\) (12300). PPC: R\(_f\) (A) 0.05, R\(_f\) (B) 0.67, R\(_f\) (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\(_2\)O (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\(_2\)O several times. The residue was recrystallized from EtOH to give 4, mp 180—181°C, in a yield of 110 mg (50%). Anal. Calcd. for C\(_{14}\)H\(_{28}\)N\(_4\)O\(_{6}\): C, 44.56; H, 4.01; N, 25.99. Found: C, 44.56; H, 3.82; N, 25.81. UV: \(\lambda_{max}^{nm} 248.5\) nm. IR: v\(_{max}^{nm}\) 2125 cm\(^{-1}\) (N3). The sample was identical with that synthesized previously\(^{15}\) by criteria of R\(_f\) 0.42 in TLC (CH\(_2\)Cl\(_2\)-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) (98 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\(_3\) sol. and H\(_2\)O, and dried over MgSO\(_4\). Evaporation of the solvent gave 5 as a hard syrup. UV: \(\lambda_{max}^{nm} 251\) (shoulder) 257, 262.5 nm; \(\lambda_{max}^{nm} 250\) (sh), 256, 262; \(\lambda_{max}^{nm} 251\) (sh), 257, 262.5 TLC (CH\(_2\)Cl\(_2\)-EtOH, 15:1); R\(_f\) 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°C, 5 ml). The tube was heated at 100°C for 4 hr. The reaction mixture was evaporated in vacuo to a syrupy residue, which was crystallized from H\(_2\)O to give 2'-azido-2'-deoxyadenosine, mp 208—210°C, in a yield of 30.2 mg (54%). This sample was identical with an authentic 2'-azido-2'-deoxyadenosine\(^{15}\) by criteria of mixed mp test, UV absorp-

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\(_2\)O (84:16); B, isoPrOH-conc NH\(_4\)OH-H\(_2\)O (7:1:2); C, n-BuOH-AcOH-H\(_2\)O (5:2:3). TLC was performed in Kieselgel HF-254 plates.
tion properties ($\lambda_{\text{max}}^{\text{MeOH}}$ 257 nm, $\lambda_{\text{max}}^{\text{EtOH}}$ 257, $\lambda_{\text{max}}^{\text{H}2\text{O}}$ 259) and $R_f$'s in paper chromatography: $R_f$ (A) 0.20, $R_f$ (B) 0.40. 

N'-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 dimethyline (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 60% estimated by optical density. UV: $\lambda_{\text{max}}^{\text{MeOH}}$ 275 nm, $\lambda_{\text{max}}^{\text{EtOH}}$ 268.5, $\lambda_{\text{max}}^{\text{H}2\text{O}}$ 273.5. IR: $\nu_{\text{max}}$ 2100 cm$^{-1}$ (N$_2$). TLC (CHCl$_3$-EtOH, 7:1): $R_f$ 0.59.

N'-Dimethyl-2'-amino-2'-deoxyadenosine (7) —— N'-Dimethyl-2'-azido compound (6) (obtained as above from 0.6 mmol of 4) was dissolved in a mixture of H$_2$O (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$_2$O, the filtrate and washings were combined and evaporated in vacuo. A glass was obtained in a yield of 240 mg (ca. 50%). UV: $\lambda_{\text{max}}^{\text{MeOH}}$ 274.5 nm, $\lambda_{\text{max}}^{\text{EtOH}}$ 266.5, $\lambda_{\text{max}}^{\text{H}2\text{O}}$ 274.5. PPC: $R_f$ (B) 0.71, $R_f$ (C) 0.66. TLC (CHCl$_3$-EtOH, 3:1) $R_f$ 0.18. These values were similar to those reported previously.9

N'-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 45%, estimated by optical density. UV: $\lambda_{\text{max}}^{\text{EtOH}}$ 266.5 nm, $\lambda_{\text{max}}^{\text{H}2\text{O}}$ 263, $\lambda_{\text{max}}^{\text{MeOH}}$ 266. IR: $\nu_{\text{max}}^{\text{CHOH}}$ 2110 cm$^{-1}$ (N$_2$). TLC (CHCl$_3$-EtOH, 5:1): $R_f$ 0.59.

N'-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$_2$O (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$_2$O. The filtrate and washings were combined and evaporated to a residue. The residue was taken up in a small amount of H$_2$O 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$_9$H$_6$N$_4$O$_7$2HCl-H$_2$O: 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 $\lambda_{\text{max}}^{\text{MeOH}}$ 266 (e 16300), $\lambda_{\text{max}}^{\text{EtOH}}$ 262 (18000), $\lambda_{\text{max}}^{\text{H}2\text{O}}$ 266 (16400). PPC: $R_f$ (A) 0.18, $R_f$ (B) 0.84, $R_f$ (C) 0.45. TLC (CHCl$_3$-EtOH, 3:1): $R_f$ 0.14.

6-Mercapto-9-(3',5'-di-O-acetyl-2'-azido-2'-deoxy-beta-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$_{13}$H$_{14}$N$_4$O$_7$: 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: $\lambda_{\text{max}}^{\text{EtOH}}$ 323.5 nm, $\lambda_{\text{max}}^{\text{MeOH}}$ 323, $\lambda_{\text{max}}^{\text{H}2\text{O}}$ 315. IR: $\nu_{\text{max}}^{\text{CHOH}}$ 2110 cm$^{-1}$. TLC (CHCl$_3$-EtOH, 15:1): $R_f$ 0.45.

6-Mercapto-9-(2'-azido-2'-deoxy-D-ribofuranosyl)purine (11) —— The compound 10 (80 mg) was dissolved in methanic 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$_2$O containing a small amount of Na$_2$SO$_4$. The sample colorized at 190° and melted at 212—214°. Anal. Calcd. for C$_{14}$H$_{15}$N$_4$O$_7$-2H$_2$O: 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 $\lambda_{\text{max}}$ 324.5 nm (e 23000), $\lambda_{\text{max}}$ 324 nm (23000), $\lambda_{\text{max}}$ 315 nm (22800). IR: $\nu_{\text{max}}^{\text{CHOH}}$ 2110 cm$^{-1}$ (N$_2$). PPC: $R_f$ (A) 0.92, $R_f$ (B) 0.84, $R_f$ (C) 0.89. TLC (CHCl$_3$-EtOH, 10:1): $R_f$ 0.10.

6-Methylthio-9-(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% NaSO$_4$ aq. (1.5 ml) was added. The reaction mixture was kept at room temperature for 12 hr. After the H$_2$O-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$_2$O and extracted with a mixture of n-BuOH (30 ml) and H$_2$O (30 ml). From the BuOH solution the compound 12 was obtained as a hard syrup in a yield of 58%. UV: $\lambda_{\text{max}}^{\text{MeOH}}$ 287, 292 mm, $\lambda_{\text{max}}^{\text{EtOH}}$ 287, 292. IR: $\nu_{\text{max}}^{\text{CHOH}}$ 2110 cm$^{-1}$ (N$_2$). PPC: $R_f$ (A) 0.93, $R_f$ (B) 0.94, $R_f$ (C) 0.92. TLC (CHCl$_3$-EtOH, 15:1): $R_f$ 0.35.

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