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A New Method for the Synthesis of 2′-Amino-2′-deoxyguanosine and -adenosine and Their Derivatives

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A new and simple procedure has been developed for the synthesis of 2′-amino-2′-deoxyguanosine (**9**) and -adenosine (**15**) and related compounds. An enzymatic transaminoribosylation between 2-chlorohypoxanthine (**6**) and 2′-amino-2′-deoxyuridine (**4**) afforded 9-(2-amino-2-deoxy-β-D-ribofuranosyl)-2-chlorohypoxanthine (**8**), which was chemically converted to **9** and its derivatives. 2′-Amino-2′-deoxyinosine (**7**) enzymatically prepared was also subjected to synthetic processes to give **15** and its derivative. The combination of chemical and enzymatic reactions was found to be useful for the synthesis of some sugar-modified purine nucleosides.

Keywords——2′-amino-2′-deoxyguanosine; 2′-amino-2′-deoxyadenosine; 9-(2-amino-2-deoxy-β-D-ribofuranosyl)-2-chlorohypoxanthine; 2′-amino-2′-deoxy-N^2-methylguanosine; 2′-amino-2′-deoxy-N^2-dimethylguanosine; 9-(2-amino-2-deoxy-β-D-ribofuranosyl)-2-hydrazinohypoxanthine; aminoribose-transfer reaction; transglycosylation

Since the discovery of puromycin, a nucleosidic antibiotic with a 3-amino-3-deoxyribose moiety, considerable effort has been directed toward the synthesis of a number of purine nucleosides containing 2- or 3-aminoribose.[1] Recently 2′-amino-2′-deoxyguanosine (**9**)[2] and -adenosine (**15**),[3,4] which show antitumor, antibacterial or antimycoplasmal activity, have been isolated in Japan, and these findings have aroused renewed interest in the preparation of 2′-amino-2′-deoxy purine nucleosides and in their biological properties.

The synthesis of 2′-amino-2′-deoxy purine nucleosides has so far been performed by a classical sugar-base coupling reaction,[5] by a cleavage reaction of 9-(2,3-anhydro-β-D-lyxofuranosyl)adenine,[6] by a reaction of the 2′-O-sulfonyl derivative of 9-β-D-arabinofuranosylpurines with azide ion,[7–9] and by a chemical transglycosylation.[10,11] However, these methods involving multi-step synthesis are time-consuming and troublesome, and in some cases the separation of several kinds of isomers is necessary. For these reasons, there have been few reports on the synthesis of analogs of **9** and **15**.

Previously we synthesized 9-β-D-arabinofuranosylpurines (Ara-X) by an enzymatic arabinose-transfer reaction between 1-β-D-arabinofuranosyluracil (**3**) and purine bases (Chart 1-a).[12,13] Subsequently, we searched for bacteria which could catalyze transaminoribosylation[14] between hypoxanthine (**5**) and 2′-amino-2′-deoxyuridine (**4**),[15] which is easily obtained *via* 2,2′-O-cyclouridine (**2**) from uridine (**1**), to afford 2′-amino-2′-deoxyinosine (**7**)[16] (Chart 1-b). In the present paper we report the preparation of 9-(2-amino-2-deoxy-β-D-ribofuranosyl)-2-chlorohypoxanthine (**8**), which was readily convertible to **9** and its derivatives. When a mixture of intact cells of *Erwinia herbicola* AJ 2803 as a wet paste, 2-chlorohypoxanthine (**6**) and **4** was incubated in phosphate buffer (pH 7.0) at 63°C for 20 h, compound **8** was formed. After removal of the bacterial cells, **8** was isolated by column chromatography (Diaion SK-1B pyridinium salt) in 32% yield based on **6**. Its identity was confirmed by analysis of the ultraviolet (UV) and nuclear magnetic resonance (NMR) spectra, the latter of which showed a $J_{1',2'}$ value (8.4 Hz) in good accord with those reported in the literature.[2,5,6] As is evident in the cases of **5** and **6**, it is noteworthy that **4** was a good substrate of the bacterial aminoribose-transfer reaction. Treatment of **8** with methanolic ammonia

in an autoclave at 150°C for 5 h gave **9** in 87% yield. Its physical properties were identical with those previously reported.[2,7]

Of special interest is the modification of **9**. Compound **8** was then converted to N^2-substituted guanine derivatives in the hope of obtaining new biologically active compounds. Substitution of 30% aqueous methylamine and 50% dimethylamine for ammonia in the synthesis of **9** afforded 2′-amino-2′-deoxy-N^2-methylguanosine (**10**) and -N^2-dimethylguanosine (**11**) in 70 and 43% yields, respectively. Treatment of **8** with hydrazine hydrate at 100°C for 5 min gave 9-(2-amino-2-deoxy-β-D-ribofuranosyl)-2-hydrazinohypoxanthine (**12**) in 75% yield.

Compound **15** was prepared *via* the 6-chloro derivative (**14**) from **7**. Acetylation of **7** with acetic anhydride in pyridine followed by chlorination of the resulting acetate (**13**) with

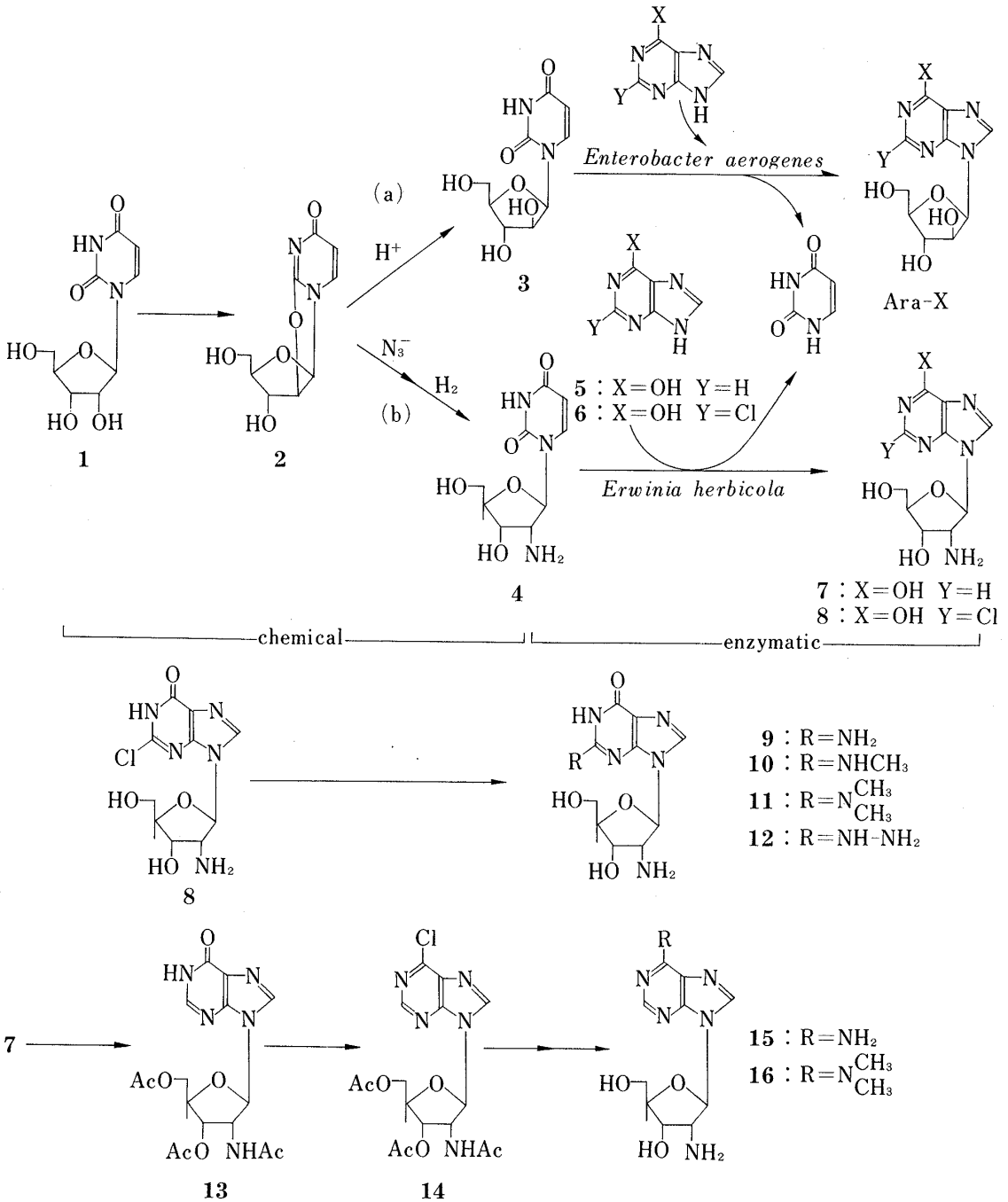

Chart 1

phosphoryl chloride gave the gummy product (**14**). This was converted by amination with ammonia and subsequent N-deacetylation with methanolic sodium methoxide to **15** (18% based on **13**). Its physical properties were identical with those reported.[5-7] Similar treatment of **13** with dimethylamine followed by sodium methoxide yielded the amorphous N^6-dimethyl derivative (**16**)[16] which could not be obtained in crystalline form. However, its structure was confirmed by the UV and NMR spectra.

Although detailed biological tests of the new compounds are still in progress, preliminary tests show that **12** has growth inhibitory activity against Hela cells and *Commamonas terigera*.

Thus, the enzymatic transglycosylation method might have wide applicability for the preparation of various sugar-modified purine nucleosides having a furanose ring. In addition, the combination of the above chemical and enzymatic reactions should lead to a variety of new and unusual purine nucleosides of biological interest.

Experimental

All melting points are uncorrected. NMR spectra were recorded on a 90 MHz Varian EM 390 spectrometer with tetramethylsilane (TMS) or sodium 2,2-dimethyl-2-silapentane 5-sulfonate (DSS) as an internal standard. UV spectra were obtained on a Hitachi 320 spectrophotometer. Silica gel chromatography was performed using Merck silica gel 60 (230—400 mesh).

9-(2-Amino-2-deoxy-β-D-ribofuranosyl)-2-chlorohypoxanthine (**8**)——A mixture of 2-chlorohypoxanthine (**6**)[17] (34 g, 0.2 mol) and 2′-amino-2′-deoxyuridine (**4**)[15] (144 g, 0.6 mol) and *Erwinia herbicola* AJ 2803 (1 kg, as a wet paste) in 20 l of 25 mM potassium phosphate buffer (pH 7.0) was incubated at 63°C for 22 h. The pH of the incubation mixture was adjusted to 4.0 with AcOH and then bacterial cells were removed by centrifugation. The supernatant was applied on a column (5×50 cm) of Diaion SK-1B (pyridinium type), which was washed with water. The product was eluted with 3% ammonia in 10% methanol–water. Fractions of 100 ml were collected. The fractions (7—17) containing only product (**8**) were pooled (eluate A). The following fractions (18—23), which contained the product, starting materials and uracil, were combined and evaporated to half the initial volume. The pH of the eluate was adjusted to 4.0 and the solution was applied on a column of Diaion SK-1B (pyridinium type) and treated as described above. The initial fractions containing **8** were collected (eluate B). The eluates A and B were combined and evaporated to dryness to afford a crude product, which was then dissolved in 150 ml of water by adding 5 N NaOH. The solution was filtered and the pH of the filtrate was adjusted to 7.0 with 2 N HCl. On standing overnight, the solution provided pure crystals in a yield of 19.8 g (32%), mp>300°C (darkens at 240°C). *Anal.* Calcd for $C_{10}H_{12}ClN_5O_4 \cdot 1/2H_2O$: C, 38.65; H, 4.21; N, 22.54. Found: C, 38.35; H, 4.16; N, 23.01. UV: $\lambda_{max}^{pH\ 1.5}$ 250 nm (ε 11700), $\lambda_{max}^{pH\ 13}$ 255 nm (ε 13200). NMR (D_2O, pD=12, uncorrected) δ: 8.15 (1H, s, H-8), 5.80 (1H, d, H-1′, $J_{1',2'}$=8.4 Hz), 4.0 (1H, q, H-2′, $J_{2',3'}$=5 Hz).

9-(2-Amino-2-deoxy-β-D-ribofuranosyl)guanine (**9**)——Compound **8** (3.1 g, 10 mmol) was sealed in an autoclave with sat. methanolic ammonia (90 ml) and then heated at 150°C for 5 h. The solution was evaporated to dryness *in vacuo* to give a residue, which was dissolved in 200 ml of water. The solution was applied on a column (3×30 cm) of Dowex 1×4 (HCO_3^-), the column was washed with water, and the product was eluted with 1 M NH_4HCO_3. The fractions containing the first peak were combined and evaporated to dryness. Co-evaporation with EtOH gave a crystalline mass, which was recrystallized from 50% aqueous EtOH to afford pure crystals (2.76 g, 87%), mp 250°C (darkens at 220°C). *Anal.* Calcd for $C_{10}H_{14}N_6O_4 \cdot 2H_2O$: C, 37.74; H, 5.70; N, 26.40. Found: C, 37.27; H, 5.44; N, 26.80. UV: $\lambda_{max}^{pH\ 1.5}$ 255 nm (ε 13000), 275 nm (sh); $\lambda_{max}^{pH\ 13}$ 255—264 nm (plateau) (ε 11700). NMR (DMSO-d_6) δ: 7.82 (1H, s, H-8), 6.38 (2H, 2-NH_2), 5.40 (1H, d, H-1′, $J_{1',2'}$=8.0 Hz).

9-(2-Amino-2-deoxy-β-D-ribofuranosyl)-N^2-methylguanine (**10**)——Compound **8** (1.0 g, 3.2 mmol) was treated with 30% aqueous methylamine -15 ml) by the same procedure as described for **9** and then heated at 150°C for 3 h. When the reaction mixture was concentrated to about 5 ml, a crystalline product precipitated. This was recrystallized from water to give pure crystals (680 mg, 70%), mp 180°C (darkens at 175°C). *Anal.* Calcd for $C_{11}H_{16}N_6O_4 \cdot 1/3H_2O$: C, 43.71; H, 5.56; N, 27.80. Found: C, 44.07; H, 5.97; N, 27.65. UV: $\lambda_{max}^{pH\ 1.5}$ 259 nm (ε 13800), 282 nm (sh); $\lambda_{max}^{pH\ 13}$ 257 nm (ε 11800), 270 nm (sh). NMR (D_2O, pD=12.5) δ: 7.89 (1H, s, H-8), 5.80 (1H, d, H-1′, $J_{1',2'}$=8.1 Hz), 2.90 (3H, s, N–CH_3).

9-(2-Amino-2-deoxy-β-D-ribofuranosyl)-N^2-dimethylguanine (**11**)——Compound **8** (560 mg, 1.8 mmol) was treated with 50% aqueous dimethylamine by the same procedure as described for **9**. The reaction mixture was concentrated *in vacuo* and the residue was crystallized from hot water (20 ml) to yield pure crystals (240 mg, 42%), mp 243°C (dec.). *Anal.* Calcd for $C_{12}H_{18}N_6O_4 \cdot 1/3H_2O$: C, 45.56; H, 5.95; N, 26.57. Found: C, 45.81; H, 5.83; N, 26.55. UV: $\lambda_{max}^{pH\ 1.5}$ 264 nm (ε 15900), 288 nm (sh); $\lambda_{max}^{pH\ 13}$ 262 nm (ε 13500), 275 nm (sh). NMR (D_2O, pD=11) δ: 7.90 (1H, s, H-8), 5.85 (1H, H-1′, $J_{1',2'}$=7.8 Hz), 3.10 (6H, s, $N(CH_3)_2$).

9-(2-Amino-2-deoxy-β-D-ribofuranosyl)-2-hydrazinohypoxanthine (12)——A mixture of **8** (500 mg, 1.61 mmol) and hydrazine hydrate (0.5 ml) was heated at 100°C for 5 min. After the reaction, water (1 ml) was added to give a clear solution. On the addition of EtOH (7 ml), crystals precipitated. The resulting crystals were collected by filtration and washed with a small amount of 50% aqueous EtOH and subsequently with EtOH to provide 370 mg (75%) of pure Crystals, mp 220°C (dec.). *Anal.* Calcd for $C_{10}H_{15}N_7O_4 \cdot 1/3H_2O$: C, 38.83; H, 5.45; N, 31.70. Found: C, 38.46; H, 5.25; N, 31.84. UV: $\lambda_{max}^{pH\ 1.5}$ 254 nm (ε 12800), $\lambda_{max}^{pH\ 13}$ 254 nm (ε 10500), 270 nm (sh). NMR (D_2O, pD=7.5) δ: 8.05 (1H, s, H-8), 5.88 (1H, d, H-1′, $J_{1',2'}$=7.8 Hz).

9-(2-Acetamido-2-deoxy-3,5-diacetyl-β-D-ribofuranosyl)hypoxanthine (13)——Compound **7** (5.34 g, 20 mmol) and acetic anhydride (60 ml) were added to 300 ml of pyridine and the mixture was warmed at 45°C for 16 h with stirring. After the solvent had been removed *in vacuo*, addition and evaporation of EtOH were repeated. The resulting residue was dissolved in 50 ml of $CHCl_3$ and the solution was applied to a column (4×30 cm) of silica gel. The product was eluted with $CHCl_3$–EtOH (8: 2, v/v), then the fractions containing the desired product were combined and concentrated to give a gummy product. This was crystallized from EtOH to give colorless **13** (4.65 g, 57.9%), mp 214—217°C. *Anal.* Calcd for $C_{16}H_{19}N_5O_7 \cdot 1/2H_2O$: C, 47.76; H, 5.00; N, 17.40. Found: C, 47.89; H, 4.98; N, 17.36.

9-(2-Acetamido-2-deoxy-3,5-diacetyl-β-D-ribofuranosyl)-6-chloropurine (14)——A mixture of **13** (2.4 g, 6 mmol), $POCl_3$ (13 ml) and diethylaniline (0.8 ml) was refluxed for 5 min with exclusion of moisture. The reaction mixture was concentrated *in vacuo* to give a residue, which was added to ice-water (50 ml) with stirring. After the product had been extracted with $CHCl_3$ (50 ml×4), the $CHCl_3$ layer was washed with water and dried over anhydrous Na_2SO_4. Removal of the solvent by evaporation *in vacuo* gave a syrup in a yield of 1.2 g (48%). The syrup thus obtained was employed in the next reaction.

9-(2-Amino-2-deoxy-β-D-ribofuranosyl)adenine (15)——Compound **14** (200 mg, 0.48 mmol) was added to 20 ml of methanol saturated with ammonia at 0°C, and the mixture was heated in an autoclave at 120°C for 3 h. The mixture was cooled, and the solvent was evaporated off *in vacuo*. The residue was dissolved in 5 ml of water, and the solution was applied to a column (1.6×80 cm) of Sephadex G-10. The nucleosidic compound was eluted with water and fractions of 10 ml were collected. The desired fractions (10—20) were combined and evaporated to dryness *in vacuo*. The residue, after being dried over P_2O_5 *in vacuo*, was dissolved in 10 ml of absolute MeOH with 1 N NaOMe and the solution was refluxed for 8 h. The reaction mixture was neutralized with 50% AcOH and evaporated to dryness *in vacuo*. The residue was dissolved in 4 ml of water and the solution was applied on a column of Sephadex G-10 and treated as described above. The desired fractions (10—17) were combined and evaporated to dryness *in vacuo*. Addition and evaporation of EtOH were repeated to give crystals in a yield of 47 mg (37%), mp 194—198°C. *Anal.* Calcd for $C_{10}H_{14}N_6O_3$: C, 45.09; H, 5.30; N, 31.56. Found: C, 45.13; H, 5.12; N, 31.23. UV: $\lambda_{max}^{pH\ 1.5}$ 256 nm; $\lambda_{max}^{pH\ 13}$ 259 nm. NMR (D_2O, pD=10) δ: 8.30 (1H, s, H-8), 8.15 (1H, s, H-2), 5.85 (1H, d, $J_{1',2'}$=8.1 Hz).

9-(2-Amino-2-deoxy-β-D-ribofuranosyl)-N⁶-dimethyladenine (16)——A solution of **14** (200 mg, 0.48 mmol) in 20 ml of 50% methanolic dimethylamine was heated in an autoclave at 120°C for 3 h. After removal of the solvent *in vacuo*, the mixture was worked up in the same manner as described for **15**. An amorphous substance was obtained in a yield of 38 mg (27%). UV: $\lambda_{max}^{pH\ 1.5}$ 268 nm; $\lambda_{max}^{pH\ 13}$ 274 nm. NMR (D_2O–DMSO-d_6) δ: 8.40 (1H, s, H-8), 8.31 (1H, s, H-2), 6.00 (1H, d, H-1′, $J_{1',2'}$=8.0 Hz), 3.45 (6H, s, $N(CH_3)_2$). The other physical properties were identical with those reported.[16,18]

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