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Studies of Nucleosides and Nucleotides. LXXIX.[1] Purine Cyclonucleosides. (37). The Total Synthesis of an Antibiotic 2′-Amino-2′-deoxyguanosine[2]

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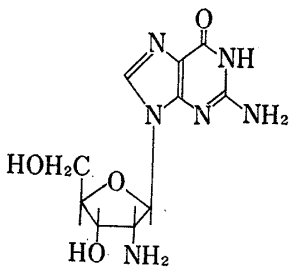
An antibiotic 2′-amino-2′-deoxyguanosine (I) was synthesized chemically from a guanine 8,2′-O-cyclonucleoside (II), which could be obtained from guanosine. Compound (II) was converted to N^2,3′,5′-triacetyl derivative (III), which was subjected to opening of the anhydro linkage with liq. H_2S in pyridine. Resulting 8-mercaptoarabinofuranosylguanine (IV) was dethiolated and mesylated to give N^2,3′,5′-triacetyl-2′-mesylarabinosylguanine (VI). Deprotection of VI and reprotection with tetrahydropyranyl groups gave compound VIII. Reaction of the compound VIII with sodium azide in acetamide at 210° for 10 min gave 2′-azido compound (IX). The compound IX was deprotected to give 2′-azido-2′-deoxyguanosine (X). Raney nickel catallyzed hydrogenolysis of X gave 2′-amino-2′-deoxyguanosine, which was proved to be identical with a sample of the antibiotic.

Keywords——ring opening of 8,2′-O-cycloguanosine; 3′,5′-disubstituted arabinosylguanine; nucleophilic inversion; UV; NMR; paper chromatography; TLC; 2′-deoxy-2′-azidoguanosine; 2′-deoxy-2′-aminoguanosine

Aminonucleoside antibiotics such as puromycin are known as inhibitors of protein biosynthesis and widely used for elucidation of the mechanism[4] of the protein synthesis. All these antibiotics have exclusively 3′-amino-3′-deoxyribofuranose structure. Recently, Nakanishi, *et al.*[5] found an antibiotic from the culture broth of *Enterobacter cloacae* and elucidated its structure as 2′-amino-2′-deoxyguanosine (I).[6] Since this antibiotic is the first naturally occurring nucleoside having 2′-amino-2′-deoxyribofuranose structure, it might be worthwhile to synthesize this compound chemically. We have studied several ways to synthesize 2′-amino-2′-deoxyribofuranosyl nucleosides[7] starting from 8,2′-O-cyclonucleosides,[8] which is obtainable from the naturally occurring adenosine.[9,10] Several years ago we have developed a versatile method to synthesize 8,2′-O-cyclonucleoside of guanosine[11] (II) which led to a new method for the synthesis of 9-β-D-arabinofuranosylguanine.[12]



I

Chart 1

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2) A part of this study has been reported briefly: M. Ikehara, T. Maruyama, and H. Miki, *Tetrahedron Lett*, **1976**, 4485.
3) Location: *133–1 Yamadakami, Suita 565, Japan.*
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5) T. Nakanishi, F. Tomita, and T. Suzuki, *Agr. Biol. Chem.*, **38**, 2465 (1974).
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We first started with the reaction of 8,2′-O-cycloguanosine (II) with acetic anhydride in pyridine. The resulting N^2,3′,5′-O-triacetyl compound (III) was obtained as pale yellow needles of mp 147—149° in a yield of 66%. The compound III was characterized as showing correct elemental analysis, ultraviolet spectrum (UV) λ_{max} at 276 nm and signals in nuclear magnetic resonance (NMR) at 11.91 and 11.71 δ corresponding to N^1- and N^2-H, respectively. Triacetylcycloguanosine (III) was heated with H_2S in pyridine at 90—95° for 15 hr to give N^2,3′,5′-triacetyl-8-mercaptoarabinosylguanine (IV), mp 232—235° in a yield of 96%. UV absorption maximum at 300 nm suggests the 8-mercaptoguanine structure. This opening reaction of the anhydro linkage proceeded in more drastic condition without the acetyl group at N^2 position.[13]

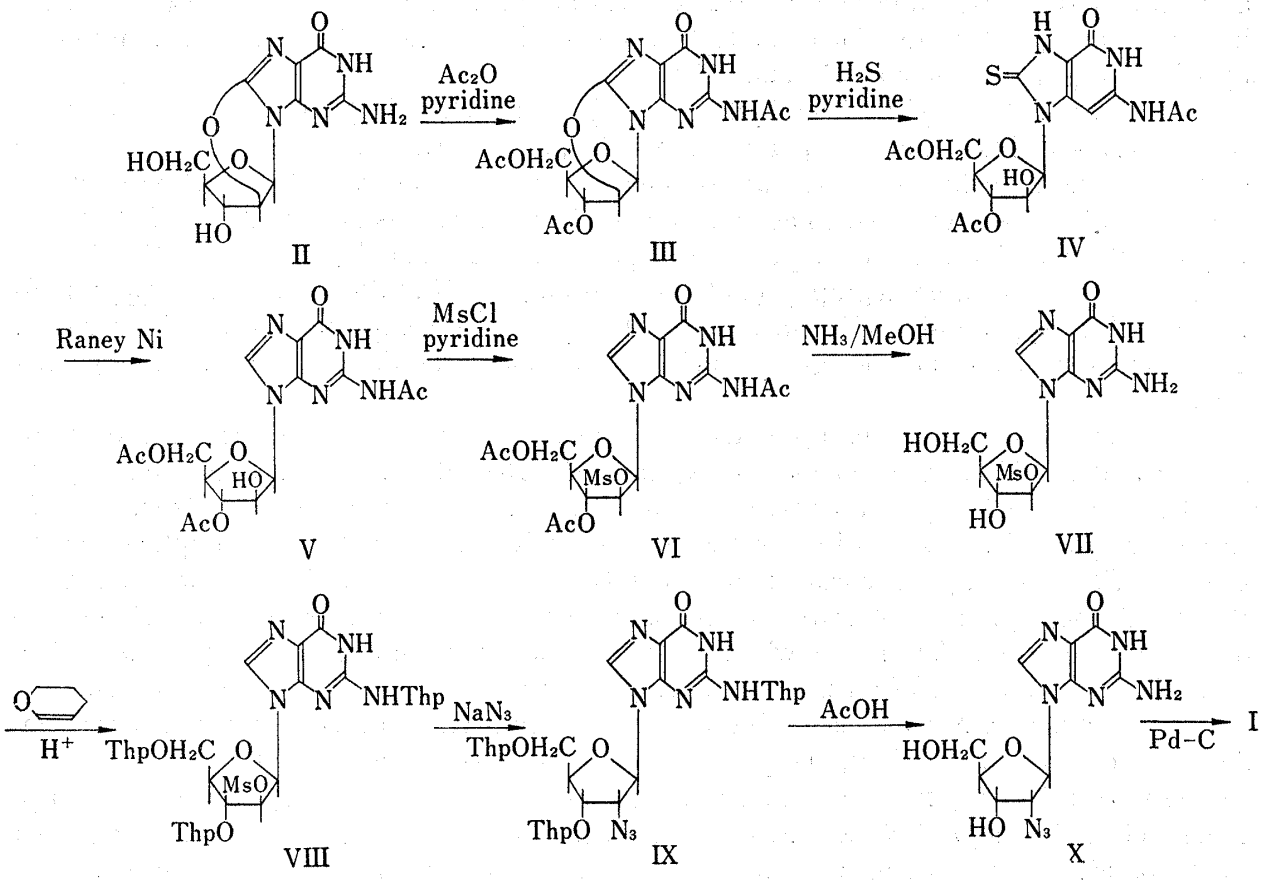

Chart 2

The compound IV was then treated with Raney nickel in refluxing alcohol to remove the 8-thiol function. N^2,3′,5′-Triacetylarabinofuranosylguanine (V) was obtained together with 3′,5′-di-O-acetyl compound, which might be formed by a specific deacylation of N-acyl group caused by refluxing in alcohol.[14,15] When the Raney nickel treatment was performed in dioxane–H_2O, the compound V was obtained in a yield of 87%. The structure was supported by its UV absorption spectra closely resembled those of N^2,3′,5′-triacetylguanosine.

As found in the case of adenosine,[2,16] 2′-OH of V in the arabinosyl configuration was attempted to invert by the mesylation and the attack with azide anion. The compound V

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was mesylated first as usual using mesyl chloride in pyridine and the resulting 2′-O-mesyl compound (VI) was obtained in a yield of 56%. The introduction of a mesyl group was confirmed by an infrared (IR) band at 1175 cm^{-1}, a signal in NMR at 3.15 δ, together with elemental analysis. The compound VI was deacetylated by treatment with methanolic ammonia to give 2′-mesyl-9-β-D-arabinosylguanine (VII) in a yield of 84%. This compound (VII) has to be protected on the 3′- and 5′-OH by non-participating tetrahydropyranyl groups, because neighboring 3′-OH group will participate to form a down epoxide at 2′,3′-position, which will give rise to a xylo-type 3′-azido compound as was found in the case of adenine nucleosides.[17] When the compound VII was treated with 2,3-dihydropyrane in DMF containing dry HCl,[18] the resulting compound had UV absorption maximum at 276 nm suggesting a cleavage of the guanine ring. Then the catalyst was changed to *p*-toluenesulfonic acid[19] and N^2,3′,5′-O-tri-(tetrahydropyranyl)-2′-O-mesylarabinosylguanine (VIII) was obtained in a yield of 64%. Introduction of a tetrahydropyranyl group to 2-NH_2 position was suggested by UV absorption maxima at 255 and 275 nm.

When the 2′-O-mesyl-tri-(tetrahydropyranyl) compound (VIII) was heated at 150° with sodium azide in DMF for a long time, the only product was 3′,5′-di-O-tetrahydropyranyl compound and no azide derivative was obtained. In order to elevate the reaction temperature, the solvent was changed to acetamide and the mixture was heated at 210° for 10 min. A prolonged reaction caused complete decomposition of the starting material. Usual work up and thin-layer chromatography (TLC) separation gave N^2,3′,5′-O-tri(tetrahydropyranyl)-2′-azido-2′-deoxyguanosine (IX) in a yield of 18%. This compound showed IR band at 2100 cm^{-1} assigned to the azide group and UV absorption properties resembled those of guanosine.

The tetrahydropyranyl group of IX was removed by treatment with acetic acid at 50° for 2 hr and 2′-azido-2′-deoxyguanosine (X), mp 185—192° (dec. 230°) was obtained in a yield of 40%. UV and IR spectra as well as elemental analysis data showed the structure of X to be correct. Direct comparison of the compound X with an authentic sample[20] showed the same *Rf* values in paper chromatography in two solvent systems. Thus, it was confirmed that the 2′-O-mesyl group of arabinosyl configuration in VIII was inverted to an azido group of ribo configuration. The reason why this inversion was more difficult in the guanine nucleoside relative to the case of adenine counterpart may be interpreted by sterical effects.

The compound X was then subjected to palladium charcoal catalyzed hydrogenation. After removal of the catalyst, the product 2′-amino-2′-deoxyguanosine (I) was obtained as colorless crystals of mp 250—252° (dec.) in a yield of 65%. This sample showed UV absorption spectrum having $\lambda_{max}^{H_2O}$ 252 and 275 nm (shoulder) and *Rf* values identical to those reported by Nakanishi, *et al.*[5] Elemental analysis also supported the structure to be correct. The fact that the precursor of the compound I, 2′-azido-2′-deoxyguanosine obtained as above was identical to that obtained by cleavage of a cyclonucleoside,[20] suggested the ribo configuration of the carbohydrate moiety of the compound I. Furthermore, the synthetic route for obtaining I is mechanistically similar to that utilyzed in the case of 2′-deoxy-2′-aminoadenosine[2] supporting the above conclusion.

Direct comparison of the compound I synthesized as above with a sample of naturally obtained antibiotic 2′-amino-2′-deoxyguanosine showed complete identity in mixed mp test and in paper chromatography in two solvent systems. Thus, it was confirmed that the antibiotic has the structure chemically defined as 2′-amino-2′-deoxyguanosine.

The present method may be versatile to obtain various 2′-substftuted 2′-deoxyguanosine derivatives and the work along this line is in progress in our laboratory.

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Experimental[21]

N^2,3′,5′-O-Triacetyl-8,2′-O-cycloguanosine (III)——8,2′-O-Cycloguanosine (6.684 g, 23.8 mmol) was dissolved in pyridine (50 ml). To the solution tetraethylammonium hydroxide (23.8 mmol) was added and evaporated *in vacuo*. Pyridine (100 ml) was added to the residue and evaporated again *in vacuo* to remove trace of water. To the anhydrous residue pyridine (350 ml) and acetic anhydride (83 ml) were added and the mixture was kept at 45—50° for 20 hr with stirring. Checking the reaction mixture by TLC ($CHCl_3$–EtOH, 7: 1) showed two spots of *Rf* 0.28 and *Rf* 0.34 in 1: 1 ratio, but the latter disappeared after work up procedure involving evaporation of the solvent and repeated distillation with added H_2O (100 ml). The residue was recrystallized from H_2O–EtOH to give triacetyl compound, mp 147—149°, in a yield of 6.345 g (15.6 mmol, 66%). *Anal.* Calcd. for $C_{14}H_{17}N_5O_8 \cdot H_2O$: C, 45.18: H, 4.50; N, 16.47. Found: C, 45.54; H, 4.23; N, 16.23. UV: $\lambda_{max}^{50\%\ EtOH}$ 257 nm (ε, 17500), 294 (11100); $\lambda_{max}^{0.1N\ HCl}$ 256 (17700), 294 (11300); $\lambda_{max}^{0.1N\ NaOH}$ 263.5 (14500). NMR (δ): 11.91 (br, 1H, N^1–H), 11.71 (br, 1H, N^2–H), 6.54 (d, 1H, H-1′, $J_{H1'-H2'}$=6.0 Hz), 5.92 (d, 1H, H-2′, $J_{H2'-H3'}$=0 Hz), 5.37 (d, 1H, $J_{H3'-H4'}$=1 Hz), 4.57 (m, 1H, H-4′), 3.98 (m, 2H, H-5′), 2.17, 2.12, 1.87 (s, each 3H, acetyl–CH_3). TLC ($CHCl_3$–EtOH, 7: 1): *Rf* 0.28.

N^2,3′,5′-O-Triacetyl-8-mercapto-9-β-D-arabinofuranosylguanine (IV)——Triacetyl-8,2-O-cycloguanosine (III) (6.34 g, 17.6 mmol) was dissolved in pyridine (40 ml) and N_2 gas was bubbled through the solution. H_2S gas was absorbed to the solution for 30 min at −50°. The solution was heated at 90—95° for 15 hrs in a sealed tube. The tube was cooled at −50°, opened and kept at room temperature for 3 hr. H_2S was chased off by bubbling of N_2 gas for 20 min. The solvent was evaporated *in vacuo* and the residue was evaporated with added toluene. The residue was dissolved in a small amount of $CHCl_3$ and the product appeared as white precipitates upon addition of toluene. Yield was 6.60 g (15.0 mmol, 96%). Analytical sample was recrystallized from EtOH, mp 150—154° resolidified at 163° and melted again with decomposition at 232—235°. UV: $\lambda_{max}^{50\%\ EtOH}$ 230 nm (shoulder), 300; $\lambda_{max}^{0.1N\ HCl}$ 230, 298; $\lambda_{max}^{0.1N\ NaOH}$ 297. TLC ($CHCl_3$–EtOH, 6: 1): *Rf* 0.53.

N^2,3′,5′-O-Triacetylarabinofuranosylguanine (V)——8-Mercapto compound (IV) (5.45 g, 12.38 mmol) was dissolved in dioxane (250 ml) and H_2O (70 ml). Raney Ni (washed well with dioxane, 10 ml) was added to the solution and the mixture was refluxed for 1 hr with vigorous stirring. Raney Ni was filtered and washed with hot dioxane–water. The filtrate and washings were combined, evaporated *in vacuo*, the residue was dissolved in $CHCl_3$, and dried over $MgSO_4$. The $CHCl_3$ solution was poured in a mixture of ether (400 ml)–hexane (300 ml) with stirring and precipitate was collected by centrifugation. Yield was 4.385 mg (10.75 mmol, 87%). UV: $\lambda_{max}^{50\%\ EtOH}$ 257, 280 nm (shoulder); $\lambda_{max}^{1N\ HCl}$ 260; $\lambda_{max}^{1N\ NaOH}$ 262.

N^2,3′,5′-O-Triacetyl-2′-O-mesylarabinosylguanine (VI)——Triacetyl-arabinosylguanine (IV) (3.826 g, 9.38 mmol) was dissolved in pyridine (30 ml). Evaporation and addition of pyridine were repeated twice. The residue was dissolved in pyridine (50 ml) and the solution was concentrated *in vacuo* to *ca.* 35 ml. Mesyl chloride (2.77 ml) was added at 0° and the solution was ketp at −20° for 2 days. The mixture was poured in ice-water (100 ml) and evaporated *in vacuo* to a residue. Addition and evaporation of H_2O were repeated and yellowish crystalls thus obtained were recrystallized from EtOH–H_2O mixture. Pale yellow needles, mp 136.5—138°, were obtained in a yield of 2.658 g (5.27 mmol, 56%). *Anal.* Calcd. for $C_{17}H_{20}N_5O_{10} \cdot H_2O$: C, 40.55; H, 4.30, N, 13.66; S, 6.67. Found: C, 40.47; H, 4.40; N, 13.88; S, 6.36. IR: ν_{max}^{KBr} 1175 cm^{-1} (covalent mesylate). UV: $\lambda_{max}^{50\%\ EtOH}$ 255 nm (ε 17500), 280 (11700); $\lambda_{max}^{0.1N\ HCl}$ 255 (16600), 272 (sh, 12100): $\lambda_{max}^{0.1N\ NaOH}$ 264 (13800). NMR (δ): 8.05 (s, 1H, H-8), 7.05 and 6.61 (br, each 1H, N^1– and N^2–H), 6.33 (d, 1H, H-1′, $J_{H1'-H2'}$ =5 Hz), 5.55 (m, 2H, H-2′ and -3′), 4.35 (m, 3H, H-4′ and -5′), 2.20, 2.13 and 2.06 (s, each 3H, Ac–CH_3), 3.14 (s, 3H, Ms–CH_3).

2′-O-Mesylarabinosylguanine (VII)——Triacetyl-2′-mesyl compound (VI) (2.242 g, 4.61 mmol) was dissolved in a methanolic ammonia solution (prepared by bubbling dry NH_3 gas to anhydrous methanol at −20°, 40 ml). The solution was kept at room temperature for 10 hr and the solvent was evaporated to 25 ml. Yellow crystalls, mp 205—208° were collected by filtration. Yield was 1.401 g, (3.89 mmol, 84%). *Anal.* Calcd. for $C_{11}H_{15}O_7S$; $1/3H_2O$: C, 36.07; H, 4.04; N, 13.66; S, 6.67. Found: C, 36.47; H, 4.40; N, 13.88; S, 6.36. IR: ν_{max}^{KBr} 1178 cm^{-1} (covalent mesylate). UV: $\lambda_{max}^{H_2O}$ 252 nm (ε, 14000), 270 (sh, 10000); $\lambda_{max}^{0.1N\ HCl}$ 256 (13100), 280 (sh, 8500) $\lambda_{max}^{0.1N\ NaOH}$ 262 (12200). NMR (δ): 7.82 (s, 1H, H-8), 6.82 (br, 2H, 3′- and 5′-OH) 5.17 (t, 1H, H-2′, $J_{H2'-H3'}$=5.5 Hz), 4.50 (t, 1H, H-3′, $J_{3'H-4'H}$=5.5 Hz), 3.50—4.00 (m, 3H, H-4′ and -5′), 3.11 (s, 3H, Ms–CH_3). PPC: *Rf* (A) 0.47, *Rf* (B) 0.52.

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21) UV absorption spectra were taken with a Hitachi EPS-3T or 124 spectrophotometer and IR spectra were taken with a Hitachi EPI-G-3 spectrophotometer. NMR spectra were taken with a Hitachi R-22 spectrometer operated at 90 MHz. d_6-DMSO was used as solvent and tetramethylsilane was used as the internal reference. TLC was performed on Kieselgel HF 254 and developed with $CHCl_3$–EtOH mixture. Paper chromatography (PPC) was performed on Toyo Roshi filter paper No. 51A using following solvent systems: A, isopropanol-conc. NH_4OH–H_2O (7: 1: 2); B, *n*-butanol–acetic acid–H_2O (5: 2: 3). All melting points were uncorrected.

N^2,3′,5′-O-Tri(tetrahydropyranyl)-2′-O-mesylarabinosylguanine (VIII)——2′-O-Mesylarabinosylguanine (VII) (274 mg, 0.76 mmol) was dissolved in anhydrous DMF (6 ml). 2,3-Dihydropyrane (3.2 ml) and *p*-toluenesulfonic acid (dried over P_2O_5 for 5 hr at 60°, 392 mg, 2.88 mmol) were added to the solution, which was kept at 4° in a refrigerator for 16 hr. Triethylamine (3 ml) was added, the solvent was evaporated *in vacuo*, and the residue was taken up in $CHCl_3$ (30 ml). After washing with H_2O (10 ml), the $CHCl_3$ solution was dried over $MgSO_4$. The solvent was evaporated *in vacuo* to a concentrated solution, which was applied to TLC ($CHCl_3$–EtOH, 10: 1). The product was obtained from a band migrating at *Rf* 0.27 by extraction with 50% EtOH. Yield was 299 mg (0.49 mmol, 64%). UV: $\lambda_{max}^{50\%\ EtOH}$ 255 nm, 275 (shoulder), $\lambda_{max}^{0.1N\ HCl}$ 256, 275 (sh) $\lambda_{max}^{0.1N\ NaOH}$ 263.

N^2,3′,5′-O-Tri(tetrahydropyranyl)-2′-deoxy-2′-azidoguanosine (IX)——Tetrahydropyranyl-2′-O-mesyl compound (VIII) (165 mg, 0.35 mmol) was mixed with acetamide (1.07 g) and well-dried NaN_3 (201 mg) was added. The reaction mixture was heated at 210° for 10 min under exclusion of moisture. H_2O (20 ml) and $CHCl_3$ (20 ml) were added to the mixture and the $CHCl_3$-layer was separated. After washing with H_2O (10 ml), the $CHCl_3$ solution was dried over $MgSO_4$. The $CHCl_3$ was evaporated to give a concentrated solution, which was applied to a preparative TLC plate. A band migrating at *Rf* 0.48 ($CHCl_3$–EtOH, 10: 1) was extracted with 50% EtOH and the compound IX was obtained as a glass in a yield of 35 mg (17%). UV: $\lambda_{max}^{50\%\ EtOH}$ 255 nm, 275 (sh); $\lambda_{max}^{0.1N\ HCl}$ 256, 280 (sh); $\lambda_{max}^{0.1N\ NaOH}$ 264. IR: ν_{max}^{Neat} 2100 cm^{-1} (N_3). A compound migrating at *Rf* 0.29 in the TLC showed UV: $\lambda_{max}^{50\%\ EtOH}$ 252 nm, 275 (sh), but no N_3 band in IR spectrum.

2′-Azido-2′-deoxyguanosine (X)——The compound IX (47 mg) was dissolved in a mixture of AcOH (4 ml) and H_2O (1 ml) and heated at 50° for 2 hr. The solvent was evaporated *in vacuo* and the residue was evaporated several times with added H_2O. The residue was dissolved in EtOH (10 ml), insoluble material was filtered off, and the filtrate was evaporated *in vacuo*. The residue was recrystallized from H_2O (2 ml) and the product was obtained in a yield of 10 mg (40%), mp 185—192° (dec. at 230°). *Anal.* Calcd. for $C_{10}H_{12}N_8O_4$: C, 38.96, H, 3.92, N, 36.35. Found: C, 38.90; H, 3.94; N, 36.33. UV: $\lambda_{max}^{H_2O}$ 253 nm, 275 (sh); $\lambda_{max}^{0.1N\ HCl}$ 256, 280 (sh); $\lambda_{plateau}^{0.1N\ NaOH}$ 258—260. PPC: *Rf* (A) 0.60; *Rf* (B) 0.52. Comparison of this sample with that of Dr. Eckstein[20] in PPC showed same *Rf* values.

2′-Amino-2′-deoxyguanosine (I)——2′-Azido-2′-deoxyguanosine (X) (30 mg) was dissolved in a mixture of H_2O (15 ml) and AcOH (3 ml). To the solution 10% Pd charcoal (45 mg) was added and H_2–gas was bubbled through with vigorous stirring. The catalyst was removed by filtration and washed with dioxane-H_2O mixture. The filtrate and washings were combined and evaporated *in vacuo*. Recrystallization of the residue from H_2O gave colorless needles, mp 250—252°, in a yield of 17.7 mg (65%). *Anal.* Calcd. for $C_{10}H_{14}N_6O_4$: C, 42.55, H, 5.00; N, 29.78. Found: C, 42.46; H, 5.12; N, 29.52. UV: $\lambda_{max}^{H_2O}$ 252 nm (ε, 13000), 270 (sh, 9200); $\lambda_{max}^{0.1N\ HCl}$ 256 (11300), 280 (sh, 8000); $\lambda_{plateau}^{0.1N\ NaOH}$ 256—262 (11000). NMR (δ): 7.82 (s, 1H, H-8), 6.38 (s, 2H, NH_2-2), 5.44 (d, 1H′, $J_{H1'-H2'}$=8 Hz). 4.99 (s, 1H, OH-5′), 3.38—4.0 (m, 5H, H-2′, -3′, -4′ and -5′), 3.26 (s, 2H, NH_2-2′). PPC: *Rf* (A) 0.25. *Rf* (B) 0.28. This compound was revealed by ninhydrine spray as violet spots on PPC. Direct comparison with a sample of authentic antibiotic[5] showed complete identity in PPC and UV absorption spectra.

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