2′-Deoxy-7-(hydroxymethyl)-7-deazaadenosine: A New Analogue to Model Structural Water in the Major Groove of DNA§

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Abstract: A novel deoxynucleoside, 2′-deoxy-7-(hydroxymethyl)-7-deazaadenosine, was synthesized with the intent of using the analogue to mimic the role of "structural" waters in the major groove of DNA. The target compound was synthesized in four steps starting from 2-((ethoxymethylene)amino)-5-bromo-1-(2′-deoxy-3′,5′-di-*O*-*p*-toluoyl-*β*-D-*erythro*-pentofuranosyl)pyrrole-3,4-dicarbonitrile. The structure was characterized by X-ray diffraction and proton NMR spectroscopic analyses. The crystal structure shows an intramolecular hydrogen bond between an amino proton on N6 and the oxygen of the hydroxymethyl group. When superimposed onto particular adenines in the structure of the tryptophan (trp) repressor/operator complex, the analogue places the oxygen of the hydroxymethyl group very near the oxygen contributed by the water in the protein/DNA complex. This analogue may be useful for probing the role of structural waters in other specific protein/DNA complexes and in DNA bending.

Introduction

Water is thought to play an important role in the specific interactions between proteins and deoxyribonucleic acids and in DNA bending dynamics. Several structures of proteins complexed with their target DNA sequences, determined both by X-ray diffraction[1a−e] and NMR[1f] spectroscopic analyses, show "fixed" water molecules acting as bridges between amino acids and hydrogen bonding sites on the target deoxyribonucleic acid. A general structural motif for these water-mediated contacts with adenine is hydrogen bonding to the N7 of the purine ring and the exocyclic amino group (Figure 1A). In addition, molecular modeling predicted similar "structural" water molecules bridging specific hydrogen bond donors and acceptors in $d(A)_5$ tracts in DNA.[2] These waters were postulated to contribute to the bending of DNA containing such poly $d(A)_n$ tracts.

To examine these questions, we synthesized a 7-deazapurine analogue, 4-amino-7-(2′-deoxy-*β*-D-ribofuranosyl)-5-(hydroxymethyl)pyrrolo[2,3-*d*]pyrimidine {2′-deoxy-7-(hydroxymethyl)-7-deazaadenosine (hm^7c^7dA) (**1**)}, and characterized its structure by X-ray diffraction and NMR analyses. Modeling this analogue into existing structures indicates that it will place an oxygen atom very close to the position oxygen occupies in the "fixed" waters of several protein/DNA structures[1a−f] or of modeled structures of bent DNA.[2] Our ultimate aim is to incorporate the analogue into oligodeoxyribonucleotides and to test its effects on the postulated interactions.

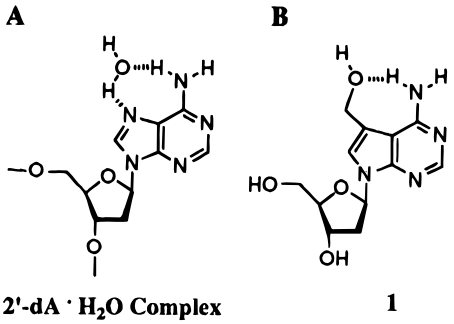

Figure 1. (A) Water-mediated contact with 2′-deoxyadenosine residue (see text) and (B) hm^7c^7dA, water-mediating mimic.

Results and Discussion

Synthesis. The synthetic strategy is based on previously synthesized 7-deazaadenosine compounds,[3,4] such as toyocamycin[3a] (Scheme 1). We synthesized the title compound **1** in four steps starting with the previously reported *β* anomer of glycosylated pyrrole **2**.[3a] Previously, cyclization using NH_3/MeOH provided the pyrrolo[2,3-*d*]pyrimidine ring system along with deprotection of the 5′ and 3′ hydroxyls.[3a] Minor modification to this method by reducing the temperature to 4 °C and the time of reaction to under 2 h yielded the desired cyclized product **3** without significant deprotection of the 5′ and 3′ hydroxyls. The progress of the reaction was followed by thin layer chromatography (TLC) (2% MeOH/CH_2Cl_2 $R_f = 0.30$), and the reaction was stopped before the 5′ and 3′ toluoylic esters were cleaved. Debromination to **4** was cleanly accomplished with Pd/C in dioxane with triethylamine and H_2 at 50 °C.[5] The nitrile **4** was reductively cleaved to the aldehyde using Raney

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(1) (a) Shakked, Z.; Guzikevich-Guerstein, G.; Frolow, F.; Rabinovich, D.; Joachimiak, A.; Sigler, P. B. *Nature* **1994**, *368*, 469−473. (b) Zhang, H.; Daqing, Z.; Revington, M.; Lee, W.; Jia, X.; Arrowsmith, C.; Jardetzky, O. *J. Mol. Biol.* **1994**, *238*, 592−614. (c) Lin, C. H.; Beale, J. M.; Hurley, L. H. *Biochemistry* **1991**, *30*, 3597−3602. (d) Clark, K. L.; Halay, E. D.; Lai, E.; Burley, S. K. *Nature* **1993**, *364*, 412−420. (e) Feng, J.; Johnson, R. C.; Dickerson, R. E. *Science* **1994**, *263*, 348−355. (f) Qian, Y. Q.; Otting, G.; Wüthrich, K. *J. Am. Chem. Soc.* **1993**, *115*, 1189−1190.

(2) (a) Teplukhin, A. V.; Poltev, V. I.; Jernigan, R. L.; Zhurkin, V. B. *Biophys. J.* **1995**, *68*, a357 [abstract]. (b) Teplukhin, A. V.; Zhurkin, V. B.; Jernigan, R. L.; Poltev, V. I. *Mol. Biol.* (*Engl. transl.*) **1996**, *30*, 75−84.

(3) (a) Bhattacharya, B. K.; Rao, T. S.; Revankar, G. R. *J. Chem. Soc., Perkin Trans. 1* **1995**, no. 21, 1543−1550. (b) Ramasamy, K.; Robins, R. K.; Revankar, G. R. *Tetrahedron* **1986**, *42*, 5869−5878.

(4) Swayze, E. E.; Shannon, W. M.; Buckheit, R. W.; Wotring, L. L.; Drach, J. C.; Townsend, L. B. *Nucleosides Nucleotides* **1992**, *11*, (8), 1507−1527.

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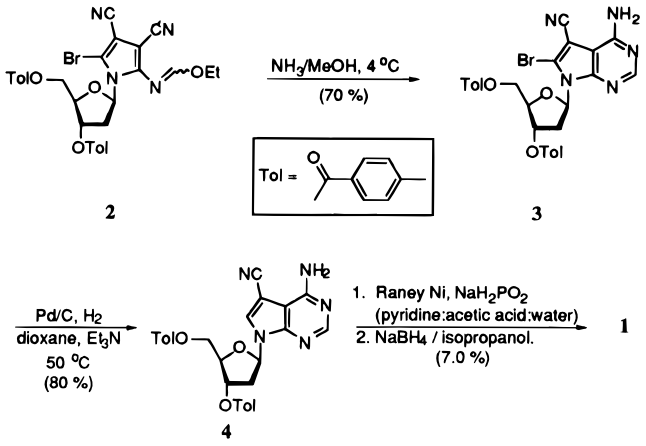
Scheme 1



Ni and sodium hypophosphate in a buffered solution (pyridine/acetic acid/water 2:1:1).[6] The aldehyde was reduced, and the toluoyl protecting groups were removed using an excess (4 equiv) of sodium borohydride.[7] Nucleoside **1** was isolated in the aqueous layer after extraction of unreacted starting material and byproducts with ethyl acetate. After the solution was concentrated under reduced pressure, compound **1** and the associated inorganic salts were slurried with methanol (two times for 30 min) and filtered to remove the inorganic salts. The compound was further purified by column chromatography and recrystallization. The low yield (7.0%) for the last step may be attributed to the multiple manipulations necessary to obtain pure product. The yield of the nucleoside, *per se*, might be increased if the reduction and deprotection were performed separately.

Structural Determination. The structure of hm^{7}c^{7}dA was determined by X-ray diffraction analysis (Figure 2). The structure revealed a possible intramolecular hydrogen bond between the amino and hydroxymethyl groups. The distance between the exocyclic amino group hydrogen (H62) and the oxygen (O11) of the hydroxymethyl group is 2.18(±0.10) Å with an angle of 157(±7)°. This distance is close enough to allow hydrogen bonding between the hydroxymethyl group and the exocyclic amino group. An extensive hydrogen-bonding network exists within the unit cell. The hydroxyl group (O11, H11) is involved in other hydrogen bond interactions: the N3 nitrogen of another nucleoside monomer and a water molecule.[8] (Table 1 lists distances and angles for all hydrogen bonds involving the amino (H61, H62) and hydroxymethyl (O11, H11) groups.) This extensive hydrogen-bonding network may contribute to the observed conformation.

Preliminary studies of the interaction between the hydroxymethyl group and the amino group in solution were performed by proton NMR spectroscopy. The water-exchangeable proton spectrum of hm^{7}c^{7}cA revealed neither a downfield shift nor a splitting of the amino protons (H61 and H62), suggesting that interaction between the hydroxymethyl group and the amino protons is transient on the NMR time scale.[9] However, the residence half-life of the exocyclic amino protons from hm^{7}c^{7}dA was about half that of the corresponding amino protons

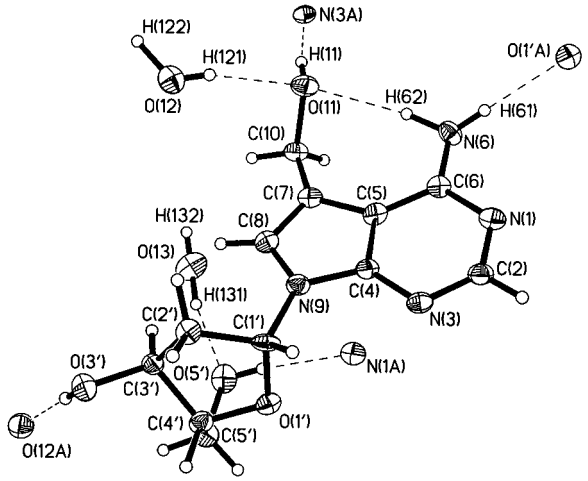


Figure 2. SHELXTL (Siemens, 1994) plot showing 35% probability ellipsoids for non-H atoms and circles of arbitrary size for H atoms. Dashed lines show H-bonding scheme. Equivalent positions O(1′A) at $^1/_2 + x$, $^3/_2 - y$, $-z$; N(3A) at x, $1 + y$, z; O(12A) at $1 - x$, $-^1/_2 + y$, $^1/_2 - z$; and N(1A) at $-^1/_2 + x$, $^3/_2 - y$, $-z$ complete the H-bonding scheme. The O(11)−N(6) distance is 3.153(8) Å. The N(6)−H(62)−O(11) angle is 157(7)°.

Table 1. Distances (Å) and Angles (deg) for Hydrogen Bond Interactions Involving the Amino Group and the Hydroxylmethyl Group

atoms	distance (Å)[b]	atoms	angles (deg)[b]
H62−O11	2.18 (0.10)	N6−H61−O11	157 (7)
H61−O1′[a]	2.24 (0.09)	N6−H62−O1′[a]	155 (7)
H121−O11	2.14 (0.07)	O12−H121−O11	161 (7)
H11−N3[a]	1.95 (0.01)	O11−H11−N3[a]	172 (7)

[a] Hydrogen bonds are with another monomer in the unit cell. [b] Errors are given in parentheses.

in 2′-deoxyadenosine. Assuming a first-order process, this small 2-fold rate enhancement may result from the hydroxyl group acting as a base to facilitate proton exchange with solvent.

The results of the structural determination by X-ray crystallography and NMR provide support for the existence of an intramolecular hydrogen bond. The proton exchange data from the solution studies suggest that the hydrogen bond between H62 and O11 exists in the absence of the other hydrogen bond interactions involving O11 that are observed in the crystal structure. If the hydrogen bond between the amino proton and the oxygen (O11) had been fixed, the amino protons signals would have split. Since this was not the case, the hydroxymethyl group is likely not restricted to one conformation in solution. The observed water-mediated contacts with adenine residues in the X-ray crystal structures of protein/DNA complexes,[1a−e] place the waters above, in, and below the plane of the purine ring. The mobility of the hydroxymethyl may allow it to adopt an energetically favorable fit when substituted into particular protein/water/adenine complexes.

We used the molecular modeling program QUANTA[10] to compare the structure of hm^{7}c^{7}dA to the corresponding adenine residues in the trp repressor/operator complex (PDB no. 1TRO) that have water-mediated contacts between the protein and the DNA.[11] The analogue was superimposed onto the relevant adenines (A_5 and A_{-7}) by aligning corresponding purine ring atoms (N1, C2, N3, C4, C5, C6, C8, and N9). The resulting superimposed structures are portrayed in Figure 3. We note

(5) Sharma, M.; Bloch, A.; Bobek, M. *Nucleosides Nucleotides* **1993**, *12*, (6), 643−648.

(6) Flanagan, D. M.; Joullié, M. M. *Synth. Commun.* **1990**, *20*, 459−467.

(7) Brown, M. S.; Rapoport, H. *J. Org. Chem.* **1963**, *28*, 3261−3263.

(8) A complete listing of all hydrogen bonds within the unit cell is included in the supporting information.

(9) The water exchange spectra were obtained in 90% H_2O/D_2O at 2 °C. The experimental details and a spectrum of the region of interest are included in supporting information.

(10) The calculations were done using QUANTA 4.1, which is available from Molecular Simulation, Inc., San Diego, CA.

(11) Otwinowski, Z.; Schevitz, R. W.; Zhang, R.-G.; Lawson, C. L.; Joachimiak, A. J.; Marmorstein, R.; Luisi, B. F.; Sigler, P. B. *Nature* **1988**, *335*, 321.

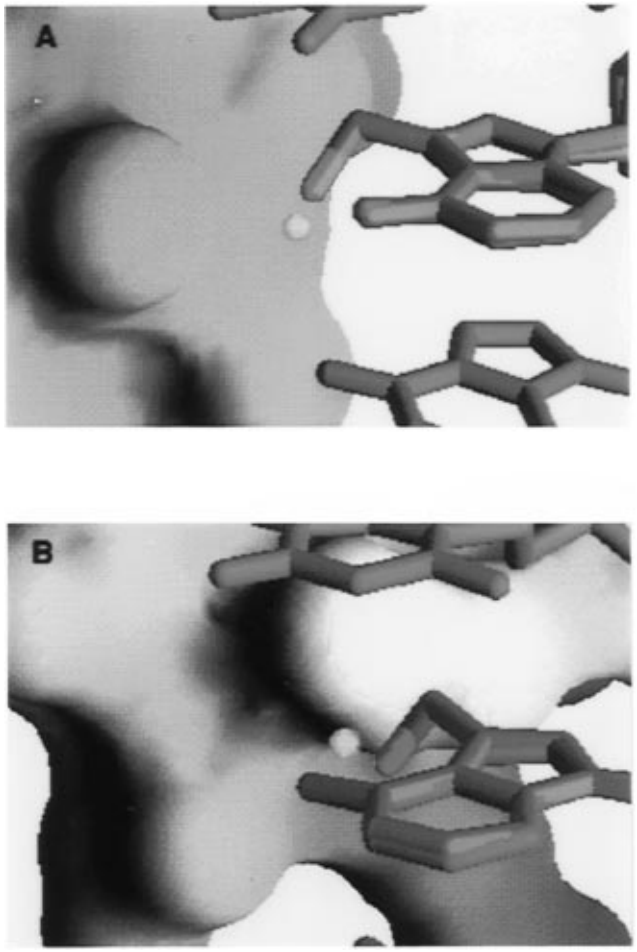


Figure 3. Modeling hm^{7}c^{7}dA into the binding pocket for water-mediated contacts to adenine residues in the trp repressor/operator complex. Protein surface calculated by Grasp (v.1.2).[13] (A) Analogue superimposed on adenine (A_5) of the operator that is recognized through a water-mediated contact with the backbone amide nitrogen of Ala 80. (B) Analogue superimposed on adenine (A_{-7}) of the operator that is recognized through a water-mediated contact with Thr83. The oxygens of the waters of interest are yellow, the DNA is green, and the analogue is aqua blue. The other strand of DNA and most of the protein were removed for clarification.

that neither the oxygen of the waters in the protein/DNA complex nor the oxygen of the hydroxymethyl group are coplanar with the purine ring.[12] The distances between the oxygen of the water and the oxygen of the hydroxymethyl group is 0.712 Å for the water mediating a contact between A_5 and the backbone nitrogen of Ala 80 and 0.835 Å for the contact mediating an interaction between A_{-7} and the hydroxyl group of Thr 83. Although the overlap is not exact, these distances are less than the resolution of the solved structure, suggesting that O11 of the analogue may be in a position suitable for mediating the hydrogen bond contacts between the protein and the DNA. A possible concern is the introduction of the slightly bulky methylene. In both relevant instances (Ala80 and Thr83) the nearest neighbor to carbon atom C10 is over 3 Å away suggesting a lack of steric clashes. The modeling used data from the X-ray diffraction analysis without energy minimization.

(12) The water associated with adenine residue A_5 is shifted out of the plane of the purine ring by 1.38 Å toward G_6 such that the water appears to be shared between A_5 and G_6. The water associated with adenine residue A_{-7} is shifted 0.671 Å toward the 3′ terminus. For hm^{7}c^{7}dA, the oxygen is 1.029 Å away from the plane of the purine ring. The plane was defined by the atoms N1, C2, N3, C4, C5, C8, and N9.

(13) Nicholls, A.; Sharp, K. A.; Honig, B. *Proteins* **1991**, *11*, 281−296.

Conclusion

We synthesized 2′-deoxy-7-(hydroxymethyl)-7-deazaadenosine. We are currently incorporating the analogue into DNA, and we plan to examine its effects on the trp repressor/operator interactions and on the bending of DNA. In summary, the structure of the analogue suggests that it may serve as a useful probe for "structural" water in adenine-containing biomolecules and their complexes.

Experimental Section

General Considerations. Most of the chemicals were purchased from Aldrich Chemical Co. and used without further purification. The 2′-deoxyadenosine for the proton exchange NMR was purchased from Sigma. ^{1}H NMR spectra were recorded on either a GE-300 (300 MHz) or Varian Unity-500 (500 MHz) spectrometer. Chemical shifts are reported relative to TMS in $CDCl_3$ or the residual DMSO peaks in DMSO-d_6. Reported melting points were taken on a Thomas Hoover melting apparatus and are uncorrected. Analytical thin layer chromatography was performed on Analtech silica gel plates with F-254 indicator. Column chromatography was carried out using Merck silica gel (50−200 μm) under 3−5 psi of air. Mass spectroscopy was performed by the University of Illinois Mass Spectrometry Laboratory, and elemental analyses were performed by the University of Illinois Microanalytical Laboratory.

4-Amino-6-bromo-7-(2′-deoxy-3′,5′-di-*O*-*p*-toluoyl-*β*-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (3). A saturated solution of ammonia in MeOH was cooled to −78 °C. To this was added 1.57 g of 2-((ethoxymethylene)amino)-5-bromo-1-(2′-deoxy-3′,5′-di-*O*-*p*-toluoyl-*β*-D-*erythro*-pentofuranosyl)pyrrole-3,4-dicarbonitrile (**2**).[3a] The solution was stirred and warmed to 4 °C. The reaction was allowed to proceed until completion as determined by TLC (R_f = 0.30 in 2% MeOH/CH_2Cl_2). The solution was then cooled to −78 °C, and the precipitation of a white solid was observed. The solid **3** was removed by filtration and used without further purification (1.04 g, 70.0%): mp 188−189 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.39 (3H, s, Ph-CH_3), 2.43 (3H, s, Ph-CH_3), 2.60 (1H, m, H2′), 3.90 (1H, m, H2′), 4.60 (1H, m, H4′), 4.65, 4.80 (2H, m, H5), 5.53 (2H, bs, NH_2), 6.01 (1H, m, H3′), 6.58 (1H, t, J = 6.6 Hz, H1′), 7.18−7.28, 7.90−7.97 (8H, m, 2 Ph), 8.25 (1H, s, H8); MS (FAB$^+$), *m/e* 592, 512 (−Br). Anal. Calcd for $C_{28}H_{24}N_5O_5Br\cdot 1/2H_2O$: C, 56.10; H, 4.20; N, 11.69; Br, 13.33. Found: C, 55.98; H, 4.16; N, 11.42; Br, 13.60.

A larger-scale synthesis of compound **3** was completed using a crude preparation of the glycosylated pyrrole **2**.[3a] The saturated ammonia/MeOH (100 mL) was cooled to 0 °C. The cooled ammonia/MeOH was transferred to a 250 mL round bottom flask that contained approximately 30 g of a crude, dried mixture containing compound **2** (cooled to 0 °C). The stirred reaction was allowed to warm to 4 °C, and the progress of the reaction was followed by TLC. Upon completion of the reaction, the mixture was concentrated under reduced pressure to a black tar. The tar was repeatedly washed with MeOH until the odor of ammonia could not be detected. The tar was dissolved in hot EtOAc and allowed to cool. After the solution was cooled to 4 °C, a precipitate formed and was collected by filtration to yield 6.0 g of a yellow solid. The filtrate was concentrated under reduced pressure and absorbed onto approximately 2.0 g of silica gel. Flash chromatography (CH_2Cl_2/MeOH, 1−5% MeOH) followed by recrystallization in acetone/ethanol (70:30) yielded 3.05 g of yellow crystals. Both crops of crystals had the same physical and spectral properties observed for the small-scale synthesis. The overall yield for the three steps, based on the starting material 2-amino-5-bromo-3,4-dicyanopyrrole, was 32% (9.05 g).

4-Amino-7-(2′-deoxy-3′,5′-di-*O*-*p*-toluoyl-*β*-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (4). A 500 mL Parr bottle was charged with 6.0 g (10.2 mmol) of compound **3**, 16 mL of triethylamine, 2.0 g of Pd/C (10%), 50 mL of dioxane, and 50 psi of H_2 gas. The solution was shaken for 4 h at 50 °C. The solution was filtered through Celite and washed two times with 50 mL of hot EtOH. The filtrate was concentrated under reduced pressure. The product was purified by flash chromatography ($CHCl_3$/EtOAc 4:1) to yield a white solid (**4**) (4.14 g, 79.9%): mp 152−154 °C; ^{1}H NMR (300 MHz,

$CDCl_3$) δ 2.43 (3H, s, Ph-CH_3), 2.44 (3H, s, Ph-CH_3), 2.71−2.83 (2H, m, H2′), 4.62−4.79 (3H, m, H4′ and H5′), 5.63 (2H, bs, NH_2), 5.72 (1H, m, H3′), 6.70 (1H, t, J = 7.7 Hz, H1′), 7.26−7.30 (4H, m, Ph), 7.68 (1H, s, H8), 7.90−7.98 (4H, m, Ph), 8.35 (1H, s, H2); MS (FAB$^+$), *m/e* 512. Anal. Calcd for $C_{28}H_{25}N_5O_5 \cdot 1/2H_2O$: C, 64.40; H, 4.73; N, 13.35. Found: C, 64.60; H, 5.03; N, 13.46.

4-Amino-7-(2′-deoxy-β-D-ribofuranosyl)-5-(hydroxymethyl)pyrrole[2,3-*d*]pyrimidine (1). A pyridine/acetic acid/water (2:1:1) buffer (200 mL) was cooled to 0 °C to which 4.0 g (9.8 mmol) of nitrile **4** was added along with 14.5 g of sodium hypophosphate monohydrate. The flask was purged with argon, and 2.35 g of Raney Ni (60% in mineral oil) was added. The reaction was heated to 55−65 °C for 6 h. The solution was cooled to room temperature and filtered through Celite. The Celite was washed two times with hot ethanol (100 mL). The filtrate was concentrated under reduced pressure. The resulting oil was taken up in 150 mL EtOAc and to this was added 200 mL of a 0.5 M solution of citric acid. The organic layer was washed one time with 200 mL of saturated sodium bicarbonate and two times with 200 mL of saturated sodium chloride. The organic layer was concentrated, leaving a tan solid. Thin layer chromatography (R_f = 0.45, 5% MeOH/CH_2Cl_2) analysis revealed an incomplete reaction. Proton NMR ($CDCl_3$) spectroscopy on the crude mixture gave a singlet peak at 9.40 ppm corresponding to the aldehyde proton. The solid was dissolved in absolute isopropyl alcohol (20 mL) and evaporated to dryness two times. The solid was dissolved in 100 mL of absolute isopropyl alcohol, and the flask was flushed with argon. Under an inert atmosphere, sodium borohydride (371.6 mg, 9.78 mmol, 4 equiv) was added and the flask was warmed to 50 °C. The solution was stirred for 30 min and then cooled to room temperature, and 10 drops of 1.0 M HCl were added slowly. The solution was evaporated to dryness and dissolved in 100 mL of EtOAc and 30 mL of 10% sodium bicarbonate solution. The organic layer was separated, and the aqueous layer was re-extracted with 20 mL of EtOAc. The aqueous layer was concentrated under reduce pressure resulting in a white solid. (The organic layer contains a mixture of partially deprotected products. None of the fully deprotected nucleoside could be observed.) This solid was crushed and then stirred with 50 mL of absolute methanol for 30 min. The inorganic salts were removed by filtration, and the procedure was repeated. To the combined filtrates was added approximately 1.5 g of silica gel, and the solvent was removed *in vacuo*. The product was filtered through silica gel (20% MeOH/CH_2Cl_2), the combined filtrates were concentrated, and the procedure was repeated. Final purification was achieved by recrystallization from methanol to yield 218.9 mg (7.05%) of **1**: mp 211−212 °C, TLC R_f = 0.55 (20% MeOH/CH_2Cl_2); λ_{max} (H_2O)/nm 274 (pH = 7), 278 (pH = 1), 270 (pH = 12); ^{1}H NMR (500 MHz/DMSO-d_6) δ 2.09 (1H, m, H2′), 2.44 (1H, m, H2′), 3.50 (2H, m, H5′1/H5′2), 3.79 (1H, m, H4′), 4.31 (1H, m, H3′), 4.57 (2H, d, J = 5.0 Hz (CH_2OH), H101, H102), 5.06 (1H, t, J = 5.49 Hz, (CH$_2$O*H*), H5′O), 5.23 (1H, d, J = 4.0 Hz, (CH$_2$O*H*), H3′O), 5.72 (1H, t, J = 5.0 Hz, (CH$_2$O*H*), H11), 6.46 (1H, dd J_{ab} = 6.0 Hz, $J_{ab'}$ = 2.4 Hz, H1′), 6.92 (2H, bs, NH_2), 7.27 (1H, s, H2), 8.04 (1H, s, H8) (hydroxyl and amino proton shifts were verified by the disappearance from the spectra of these signals when D_2O is added to the DMSO solution); HRMS (FAB$^+$) found 281.1250, calcd for $C_{12}H_{16}N_4O_4$, 281.1245. Anal. Calcd for $C_{12}H_{16}N_4O_4$: C, 51.4; H, 5.76; N, 20.0. Found: C, 51.20; H, 5.62; N, 19.61.

X-ray Structure Determination. Compound **1** was crystallized from methanol to give 2′-deoxy-7-(hydroxymethyl)-7-deazaadenosine dihydrate: MW = 316.32, $C_{12}H_{20}N_4O_6$, crystal dimensions of 0.10 × 0.14 × 0.36 mm, orthorhombic, space group $P2_12_12_1$, a = 7.0764(11) Å, b = 8.0669(13) Å, c = 24.681(4) Å, V = 1408.9(4) Å^3, Z = 4, ϱ_{calcd} = 1.491 mg/m^3, θ = 3.30−22.64°, Mo Kα radiation (λ = 0.71073 Å), and T = 198 K. Data were collected using a Siemens SMART/CCD, and the structure was solved *via* direct methods. Full-matrix least squares refinement on F^2 using SHELXTL-V5.0 converged with a final R^1 = 0.074 and wR^2 = 0.175 for 1413 observed ($I > 2\sigma(I)$) reflections. Positions and displacement parameters for water and amino H atoms were independently refined, torsion angles for idealized hydroxyl H atoms were refined, and the remaining H atoms were included as fixed idealized contributors. Isotropic U values for idealized H atoms were assigned as 1.2 times the U_{eq} of the adjacent atom.

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Note Added in Proof: The compound 5-(hydroxymethyl)-tubercidin, the ribonucleoside version of the title compound, was previously synthesized starting from the natural product sangivamycin (5-carboxamidetubercidin) using completely different chemistry. Uematsu, T.; Suhadolnik, R. J. *J. Med. Chem.* **1973**, *16* (12), 1405−1407.

Supporting Information Available: X-ray diffraction data and proton NMR data in water (8 pages). See any current masthead page for ordering and Internet access information.

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