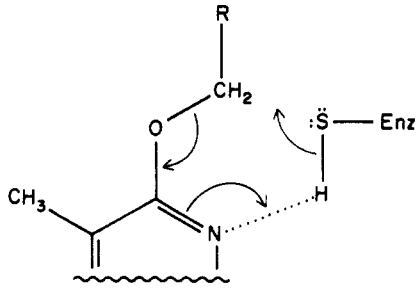

Figure 7. Proposed mechanism of the transfer of the O^4-alkyl group to the repair enzyme.

The ethyl groups of e^4dT and e^4dU display A_3X_2 patterns with $J(A,X) = 7.1$ Hz. That the methylene protons are isochronous means that they are symmetrically placed relative to the pyrimidine and in particular to the N3 ring atom. This situation obtains in the conformation revealed in the crystal state, namely the syn-periplanar and anti-periplanar orientations about the C4-O4 and O4-C8 bonds, respectively.

The $J(5,6)$ coupling constant in e^4dU is 7.5 Hz, in line with observations[16] that it is typically 0.5 Hz smaller in pyrimidines with "cytosine-like" electronic structures than in those with a 2,4-diketo structure. Following the literature,[34] Hruska and Blonski[16] attributed this decrease to a decrease in the π-bond order of the C5-C6 bond. However, as shown in Table III, the bond angles in "cytosine-like" structures (e^4dT, m^4U, and m^5araC) are significantly different from those in 2,4-diketo structures (U and dT). These differences, particularly at C5 and C6, would affect the $J(5,6)$ coupling and complicate the π bond order interpretation.

Biological Implications. In order to explain the misincorporation of guanine in replicative and transcriptive systems following DNA alkylation, Abbott and Saffhill[7] postulated a G--m^4dT base mispair involving N1(G)—H···N3(T) and N2(G)—H···O2(T) hydrogen bonds. However, their scheme shows the O-CH_3 bond in m^4dT anti-periplanar to N3-C4. This places the methyl group into a position which is unacceptably close to the C5-methyl group. Brennan et al.[9] proposed a G--m^4U base pair with the same Watson-Crick hydrogen bonds but with the N3-C4-O4-CH_3 torsion angle rotated 110° from the syn-periplanar conformation.

Alternatively, we can envisage a base pair (Figure 6) in which the alkoxy group in dT remains in its favored syn-periplanar conformation and at an acceptable distance from O6(G) (~3.5 Å). Here, only the N2—H···O2 hydrogen bond would have the normal strength, whereas the N1—H···N3 bond would be substantially weakened. On the other hand, this scheme does not suffer from the destabilizing rotation of the O-alkyl group which would increase the relative energy by ~6 kcal/mol,[20] thus counteracting the stabilization gained by a hydrogen bond. No estimates have been made of the binding strength of a G--O^4-alkyl-dT base pair, but we predict that it will be shown to be weaker than might be expected for a base pair joined by two normal hydrogen bonds.

Hydrogen bonding to N3 could also play an important role in the enzymatic repair of O^4-alkylated pyrimidine residues of DNA in prokaryotes.[35,36] The process appears to proceed in the same way as the repair of O^6-methylated guanine residues by a methyl transferase,[37] i.e., transfer of the alkyl group to a cysteinyl residue, regeneration of the parent base, and deactivation of the transferase (suicide repair[38]). Details of the mechanism we envisage are shown in Figure 7. This mechanism would represent an example of general base catalysis in which the base (N3) and the attacked center reside in the same molecule. The concerted transfer of electrons is facilitated by the syn-periplanar orientation of the O^4-alkyl group which has been found in the crystal structures of e^4dT and m^4U.

This scheme can be extended to the repair of O^6-alkylated guanines. Furthermore, hydrogen bonding at N3 would probably also facilitate the repair of O^2-alkylated pyrimidines by a DNA glycosylase.[36] It may also be significant that the O^4-alkyl base bears a structural and electronic resemblance to the 5-methylcytosine base which can play an important role in the long-term inactivation of eukaryotic genes.[39]

Acknowledgment. K.L.S. and F.E.H. acknowledge NSERC for operating support (Grants A1549 and A6434). We appreciate helpful comments of W. Fritz and Drs. N. R. Hunter and J. L. Charlton. Apart from MULTAN78,[14] all crystallographic computations were carried out with programs written by Ahmed et al.[40] Figures 1 and 5 were drawn with the ORTEP program of Johnson.[41]

Supplementary Material Available: Tables of anisotropic temperature parameters of the non-hydrogen atoms and of observed and calculated structure amplitudes (7 pages). Ordering information is given on any current masthead page.

(34) Veillard, A. *J. Chim. Phys. Phys.-Chim. Biol.* **1962**, 1056-1066.
(35) McCarthy, T. V.; Karran, P.; Lindahl, T. *EMBO J.* **1984**, *3*, 545-550.
(36) Ahmmed, Z.; Laval, J. *Biochem. Biophys. Res. Commun.* **1984**, *120*, 1-8.
(37) Olsson, M.; Lindahl, T. *J. Biol. Chem.* **1980**, *255*, 10569-10571.
(38) Walsh, C. T. *Annu. Rev. Biochem.* **1984**, *53*, 493-535.
(39) Doerfler, W. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 919-931.
(40) Ahmed, F. R.; Hall, S. R.; Pippy, M. E.; Huber, C. P. *J. Appl. Crystallogr.* **1973**, *6*, 309-346.
(41) Johnson, C. K. ORTEP II Report ORNL-5138; Oak Ridge National Laboratory: Oak Ridge, TN, 1976.

New Enzymatic Synthesis of 2′-Deoxynucleoside-2′,2′-d_2 and the Determination of Sugar Ring Flexibility by Solid-State Deuterium NMR

Siddhartha Roy,*† Yukio Hiyama,‡ Dennis A. Torchia,‡ and Jack S. Cohen†

Contribution from the Clinical Pharmacology Branch, National Cancer Institute, and Mineralized Tissue Research Branch, National Institute of Dental Research, NIH, Bethesda, Maryland 20205. *Received August 26, 1985*

Abstract: An enzymatic synthesis of nucleosides selectively deuterated in the deoxyribose ring is described. The method is illustrated with the synthesis of 2′-deoxyguanosine-2′,2′-d_2 and thymidine-2′,2′-d_2. The availability of such specifically labeled nucleosides enables the motional characteristics of the deoxyribose ring to be investigated with deuterium NMR spectroscopy in solution and the solid state.

Rapid molecular motion in macromolecules results from interconversion between conformational states of comparable energy with relatively low activation energy barriers. Information about motion can provide understanding of the accessible conformational states of a macromolecule. It is possible that such states could be used for a variety of biological functions that a protein or nucleic acid performs.

Molecular motions in macromolecules have been investigated in detail[1] and motions of various frequencies and amplitudes have

†National Cancer Institute.
‡National Institute of Dental Research.

This article not subject to U.S. Copyright. Published 1986 by the American Chemical Society

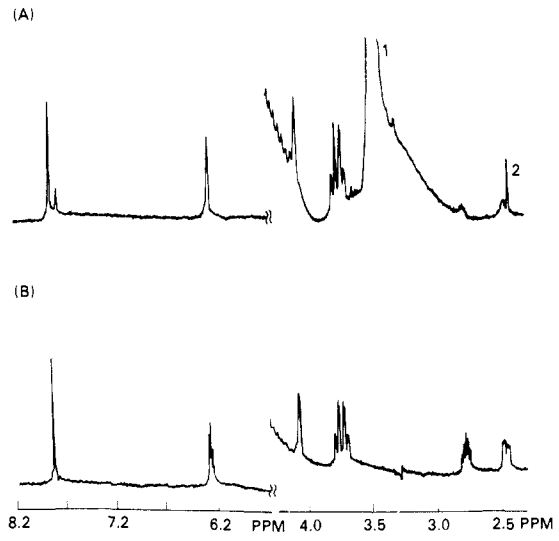

Figure 1. (A) Proton NMR spectrum of 2′-deoxyguanosine-2′,2′-d_2 at 400 MHz in ^2H_2O. The peak labeled 1 derives from excess Tris; the peak labeled 2 is an impurity; the small peak at 7.9 ppm is from residual guanine. The spectrum is an average of 16 transients. (B) Proton NMR spectrum of an authentic sample of 2′-deoxyguanosine at 400 MHz. The 3′-proton resonance is not resolved from the residual water peak. One transient.

been detected in proteins and nucleic acids.[2,3] In nucleic acids most attention has been focused on motions of the phosphate backbone and the bases.

The relaxation properties of NMR observable nuclei can be related to molecular motions. Relaxation of nuclei with spin $^1/_2$ (e.g., ^1H and ^{31}P) is related to molecular motions through internuclear (dipolar) and intranuclear (chemical shift anisotropy) mechanisms. If these two interactions are comparable in strength, it is difficult to analyze relaxation data to obtain information on motion, although in favorable cases motional characteristics can be deduced.[4] Relaxation of nuclei with spin $>^1/_2$, such as ^2H with spin = 1, is more straightforward to interpret in terms of motion,[5-7] because the dominant relaxation mechanism is the intranuclear electric quadrupole coupling. Studies of C8 deuterated base, which can be prepared by exchange with ^2H_2O under mild alkaline conditions,[8] have taken advantage of this property.

Information on the mobility of sugars in general is not available for polynucleotides, because of the lack of resolution of the sugar proton resonances and the difficulty of labeling sugars with ^2H and ^{13}C. Deuterium NMR has been used to study ring motion of deuterium-labeled proline (a five-membered cyclic amino acid) in the solid state.[7,9] A similar approach could give us information of ring mobility in ^2H-labeled nucleosides and polynucleotides. It is known from X-ray data that sugars in DNA can exist in different conformations, e.g., 2′-endo in B DNA and 3′-endo in A DNA.[10] Other sugar conformations may also be accessible for different forms of DNA. Information of sugar mobility may throw new light on conformational states and mechanisms of conformational changes in DNA. With this idea in mind, we developed a regiospecific labeling procedure of deoxyribose in nucleosides. Such labeling would also be useful in such studies as protein–nucleic acid interactions where virtually nothing is known about the role of the sugar backbones. In this paper we report a procedure for 2′-deuteration of the nucleosides 2′-deoxyguanosine and thymidine. We also report a preliminary study of the mobility of deoxyribose in these nucleosides by solid-state deuterium NMR.

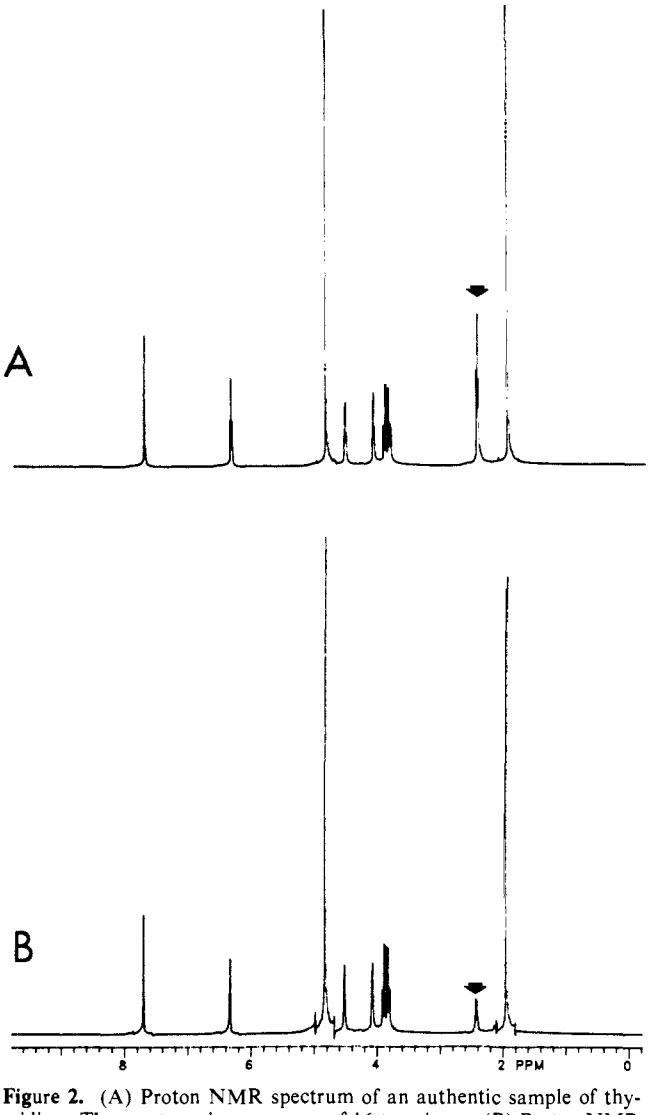

Figure 2. (A) Proton NMR spectrum of an authentic sample of thymidine. The spectrum is an average of 16 transients. (B) Proton NMR spectrum of thymidine-2′,2′-d_2. The spectrum is an average of 16 transients.

Results

Purity of the synthetic deuterated materials was judged by thin-layer chromatography and ultraviolet absorbance spectra. As described in the Experimental Section, the synthetic 2′-deoxyguanosine was chromatographed on a cellulose thin-layer plate in two different solvent system. The sample gave one spot identical with an authentic sample of 2′-deoxyguanosine. A trace amount of guanine was also detected in the sample and UV absorption spectra of the sample in acid and alkali were identical with a standard 2′-deoxyguanosine spectrum. Figure 1A shows the proton NMR spectrum of the product. Figure 1B shows the proton NMR spectrum of an authentic 2′-deoxyguanosine sample. As can be seen from the spectra peaks present at 2.8 and 2.47 ppm in the standard spectrum are greatly reduced in the synthetic sample spectrum. These protons have been assigned to the 2′- and 2″-protons of 2′-deoxyguanosine. In addition it may be observed that the peak at 6.35 ppm has become a singlet instead of a triplet in the nondeuterated sample. This is expected, since,

(1) Jardetzky, O.; Roberts, G. C. K. "NMR in Molecular Biology"; Academic Press: New York, 1981.
(2) Shindo, H. "Magnetic Resonance in Biology"; Cohen, J. S., Ed.; Wiley: New York, 1983; Vol. 2.
(3) London, R. E. "Magnetic Resonance in Biology"; Cohen, J. S., Ed.; Wiley: New York, 1980; Vol. 1.
(4) Lipari, G.; Szabo, A. *J. Am. Chem. Soc.* **1982**, *104*, 4559–4570.
(5) Wooten, J. B.; Cohen, J. S. *Biochemistry* **1979**, *18*, 4188–4191.
(6) Schramm, S.; Oldfield, E. *Biochemistry* **1983**, *22*, 2908–2913.
(7) Torchia, D. A. *Annu. Rev. Biophys. Bioeng.* **1984**, *13*, 125–144.
(8) Egan, W. *J. Am. Chem. Soc.* **1976**, *98*, 4091–4093.
(9) Sarkar, S.; Young, P.; Sullivan, C. E.; Torchia, D. A., unpublished observations.
(10) Saenger, W. "Principles of Nucleic Acids Structure"; Springer-Verglag: New York, 1984.
(11) Valentin-Hansen, P.; Bowtius, F.; Hammer-Jesperson, K.; Svensen, I. *Eur. J. Biochem.* **1982**, *125*, 561–566.

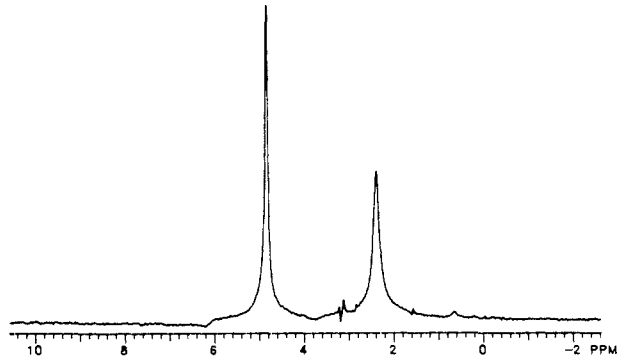

Figure 3. Solution deuterium NMR spectrum of thymidine-2',2'-d_2. The peak at 4.8 ppm is the residual HDO. Number of transients averaged were 256.

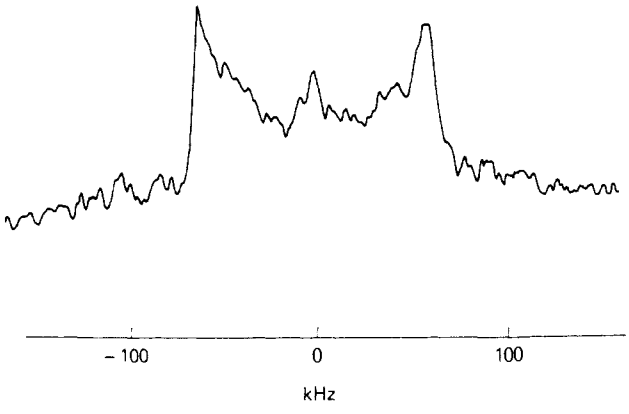

Figure 4. Solid-state deuterium NMR spectrum of 2'-deoxyguanosine-2',2'-d_2 at 38.45 MHz obtained with a quadrupolar echo sequence at 22 °C; the recycle delay time was 1 s. The spectrum is an average of 100 000 transients.

coupling of H1' (6.35 ppm) with H2' and H2'' produces the multiplicity. Proton NMR spectra of thymidine and the synthetic deuterated thymidine are shown on Figure 2. It is clear that the peak at 2.3 ppm is reduced to a great extent in the synthetic material, indicating considerable deuteration. The product also gave a UV absorbance spectrum identical with that of an authentic sample of thymidine. Figure 3 shows the solution deuterium spectrum of thymidine-2',2'-d_2. As expected it shows one deuterium peak of linewidth 8.5 Hz.

Solid-State Deuterium Powder Spectra of 2'-Deoxynucleosides-2',2'-d_2. Figure 4 shows the solid-state deuterium powder spectrum of a lyophilized sample of 2'-deoxyguanosine-2',2'-d_2. As can be seen from the spectrum, it has a quadrupolar splitting of 120 kHz, quite comparable to molecules that have rigid methylene groups. However, there are differences between the spectral parameters observed for the 2'-deoxyguanosine-2',2'-d_2 and for a completely rigid methylene moiety. First, the intensity observed with a 1-s recycle delay (Figure 4) increased by less than 25% when the recycle delay was increased to 2 s. This result shows that the average T_1 value was less than 1 s, significantly less than expected for a rigid system. Second, absorbance at 260 nm showed the presence of ca. 3.1 mg of product. Calculation of signal intensity relative to methylene groups in a sample of polyethylene of known weight indicated the presence of 1.35 mg of 2',2''-deuterated 2'-deoxyguanosine. The absence of over one-half of the NMR signal intensity shows that a significant fraction of the sample had small (<100 μs) T_2 values.[12] Third, although the quadrupolar "Pake" splitting is large (120 kHz), the line shape (Figure 4) is not well represented by an axially symmetric "Pake" powder pattern. In particular, there are shoulders just inside the perpendicular edges of the pattern.

(12) Spies, H. W.; Sillescu, H. J. *J. Magn. Reson.* **1981**, *42*, 381-409.

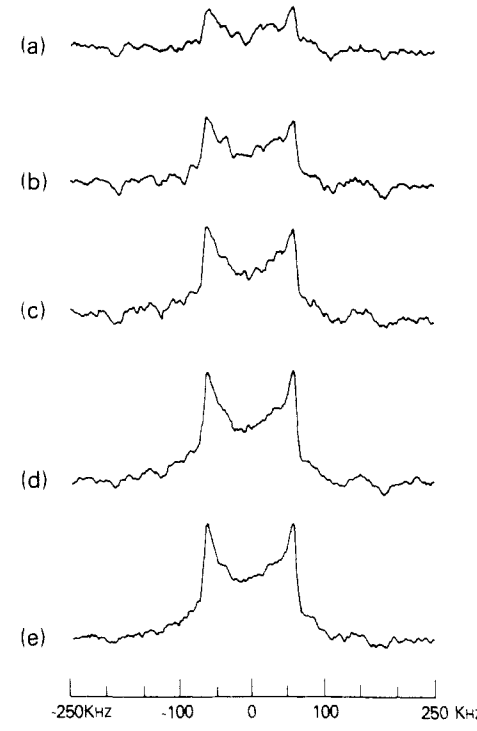

Figure 5. Solid-state deuterium NMR spectrum of thymidine-2',2'-d_2 at different recycle delay times: (a) 10 ms; (b) 40 ms; (c) 100 ms; (d) 500 ms; (e) 2 s. Number of transients were (a)-(c) 20 000; (d) 16 000; (e) 14 000.

The solid-state spectrum of thymidine is shown in Figure 5. The pattern is very similar to that of 2'-deoxyguanosine. A series of spectra at different recycle delay times shows T_1 to be approximately 0.15 s.

Discussion

Here we report a new enzymatic synthesis of selectively deuterated deoxyribonucleosides, 2'-deoxyguanosine-2',2'-d_2 and thymidine-2',2'-d_2. We have deuterated 2-deoxyribose 5-phosphate from unlabeled material by enzyme-catalyzed exchange with solvent D_2O. The enzyme used was 2-deoxyribose-5-phosphate aldolase. A possible mechanism is depicted below.

$$\mathrm{RCH(OH)CH_2CHO} \xrightarrow{\text{enzyme}/D_2O} \mathrm{RCHO + CH_2DCHO}$$

$$\mathrm{RCHO + CH_2DCHO} \xrightarrow{\text{enzyme}} \mathrm{RCH(OH)CHDCHO} \rightarrow \text{repeat}$$

Usually equilibrium in the aldolase reaction lies well toward the synthesis side and hence the yield of labeled material will be high. The next step involves conversion of labeled 2-deoxyribose 5-phosphate to 2-deoxyribose 1-phosphate by phosphopentomutase, followed by attachment of the base guanine by purine nucleoside phosphorylase. The equilibrium of the phosphopentomutase reaction lies well toward the 2-deoxyribose 5-phosphate, but the equilibrium of the nucleoside phosphorylase reaction lies well toward the nucleoside product. In order to obtain a reasonable yield of nucleoside from 2-deoxyribose 5-phosphate it is necessary to couple these reactions.

To our knowledge there has only been one 2'-deuteration procedure for deoxynucleosides reported in the literature.[13] This procedure involved reduction of 2'-derivatives of adenosine with tributyltin deuteride and is only relevant for deuteration of 2',2''-positions of deoxynucleosides. Recently, an enzymatic procedure for labeling of ribose has also been reported.[14] In contrast, the method we report here has the potential to introduce both ^{13}C and ^{2}H at any position of the deoxyribose ring of deoxyguanosine, deoxyinosine, or thymidine.

(13) Robins, M. J.; Wilson, J. S. *J. Am. Chem. Soc.* **1981**, *103*, 932-933.
(14) Parkin, D. W.; Leung, H. B.; Schramm, V. L. *J. Biol. Chem.* **1984**, *259*, 9411-9417.

Although nucleoside phosphorylase from calf thymus has strict specificity for hypoxanthine and guanosine, the *E. coli* enzyme accepts adenine as a substrate.[15] The *E. coli* enzyme is part of the deo operon and is overproduced in the CGSC-5903 strain. It can be isolated by the same procedure described in this article. The reaction synthesizes the deoxyribose ring from smaller molecules, acetaldehyde and glyceraldehyde 3-phosphate. Acetaldehyde ^{13}C and ^{2}H labeled is available commercially. Regiospecificially labeled glyceraldehyde 3-phosphate can also be made by a variety of procedures. Thus, the method has the potential to become a general method of synthesizing nucleosides with labeled deoxyribose sugars both with respect to regiospecificity and type of nucleosides.

We have also made a preliminary examination of the mobility of the sugar ring by solid-state deuterium NMR spectroscopy. The spectrum shows a component with a quadrupolar splitting of ca. 120 kHz which is approximately the same as that of rigid polymers. However, it was estimated by observing peak intensity at different recycle delay times that the average T_1 of the deuterium was less than ca. 1 s. This is considerably shorter than the T_1 (ca. 20 s) of the more rigid deuteriomethylene group of crystalline polyethylene. This indicates the presence of small amplitude motion on a time scale of $<10^{-6}$ s. Consistent conclusions can be drawn from the solid-state deuterium NMR spectrum of thymidine. The similarity of the spectrum suggests that small-amplitude motion that leads to low T_1 values may be similar in both cases and perhaps independent of the nature of the base. Motions in the same range are also consistent with partial intensity loss in the whole spectrum as well as differential loss of intensity in the central part of the spectrum. We are extending these studies to solution and further solid-state deuterium NMR measurements in order to test the hypothesis that the C3′-endo-C2′-endo sugar conformations are interconverting at an extremely rapid rate.[16,17] This deuterium labeling method should also aid in drug and protein binding studies with nucleic acids, through changes in local mobility from deuterium NMR observations.

Experimental Section

Materials. 2-Deoxyribose 5-phosphate, guanine, 2′-deoxyguanosine, glucose 1,6-diphosphate, purine nucleoside phosphorylase, alcohol dehydrogenase, and 2-deoxyribose 1-phosphate are all from Sigma Chemical Co. DE-52 was from Whatman Co. Sephadex G-10 was from Pharmacia Chemical Co. deoR$^-$cytR$^-$ *E. coli* (CGSC-5903) was a kind gift from Dr. Barbara Bachmann.

Methods. Thin-layer chromatography was performed in two different solvent systems, (a) *tert*-butyl alcohol/acetone/water/ammonium hydroxide 4:3:2:1 and (b) ethyl acetate/water/acetic acid 4:1:1.

NMR Methods. Solid-state deuterium NMR spectrum is taken on a home-built 38.45-MHz spectrometer. The 90° pulse width was 2.2 μs. Other parameters are described in the figure legends. T_1s are estimated from the intensity of the spectra at different recycle delay times.

Growth of CGSC-5903. CGSC-5903 (deoR$^-$cytR$^-$) strain was grown in a minimal medium with glycerol as carbon source. The enzyme yields are maximum under this condition. Media consisted of water (975 mL), glycerol (20 mL), KH_2PO_4 (13.6 g), $MgSO_4$ (0.2 g), $CaCl_2$, $2H_2O$ (13 mg), $Fe(NH_4)_2(SO_4)_2$, $6H_2O$ (1 mg), $(NH_4)_2SO_4$ (2 g), 1% solution of valine, isoleucine, methionine (4 mL) in 1 M HCl, and 1% thiamine hydrochloride (2 drops), titrated to pH 7.4 with NaOH (10 N). Cells were grown overnight and harvested. At typical yiedl was 3 g/L of liquid culture.

Enzyme Purification. All purifications were done at 4 °C. Enzymes were isolated from a deoR$^-$cytR$^-$ *E. coli* strain (CGSC-5903) which overproduces all deoR operon enzymes.[11] Cells (6.2 g) were suspended in 0.1 M Tris-Cl (25 mL), pH 7.6, at 4 °C containing EDTA (2 mM) and sonicated in a Heat-Systems Ultrasonics sonicator for 6 × 30 s with 5-min intervals. The cell debris was removed by centrifugation at 10 500 rpm for 30 min. Streptoymcin sulfate was added to the supernatant to make a final concentration of 1%. Precipitate obtained after centrifuging for 30 min at 10 500 rpm was discarded. A 40–65% ammonium sulfate cut was made. The ammonium sulfate cut was dissolved in minimum amount of Tris-succinate buffer (10 mM), NaCl (50 mM), and EDTA (0.2 mM), pH 6.9 and dialyzed against the same buffer for 24 h.

The dialyzate was applied on a DE-52 column (2.5 × 3.8 cm) equilibrated with Tris-succinate buffer (10 mM), NaCl (50 mM), and EDTA (0.2 mM), pH 6.9, and eluted with 1 L of the same buffer. Deoxyribose 5-phosphate aldolase elutes in this isocratic elution. A 500 mL/500 mL of NaCl (50 mM:300 mM) gradient was applied to elute phosphopentomutase and purine nucleoside phosphorylase. Enzymes elute at the end of the gradient, with nucleoside phosphorylase first and phosphopentomutase eluting later. The enzymes were pure enough at this stage to be used in the synthetic procedures described below.

Enzyme Assays. 2-Deoxyribose-5-phosphate aldolase was assayed in the dissociation direction by coupling the acetaldehyde formation with alcohol dehydrogenase and measuring the NAD^+ production at 366 nm.[11] The assay mixture consisted of 2-deoxyribose 5-phosphate (2 μmol), NADH (275 nmol), and alcohol dehydrogenase (45 μg) in 1 mL of buffer (0.05 M Tris-Cl, 0.1 mM EDTA, pH 7.61. Assayed material (10 μL) was added directly into the mixture. Phosphopentomutase reaction was assayed in the direction of 2-deoxyribose 1-phosphate production, it was coupled to guanosine production by purine nucleoside phosphorylase, and the absorbance increase at 257 nm was observed. Assay mixture consisted of glucose 1,6-diphosphate (5 nmol), guanine (128 nmol, added as saturated solution in 1 M HCl), purine nucleoside phosphorylase (1 unit) in 1 mL buffer (0.1 M Tris-Cl, 0.1 mM $Mncl_2$, pH 8.1). Phosphopentomutase was added and preincubated for 3 minutes and 2-deoxyribose 5-phosphate (4 μMoles) was added to initiate the reaction.

Deuteration of 2-Deoxyribose 5-Phosphate. Deoxyribose-5-phosphate aldolase (0.33 units) was added to 2-deoxyribose 5-phosphate solution in D_2O (7 mL, 350 μmol). The extent of the reaction was followed by proton NMR. After 2 days at room temperature 90% deuteration of the C2H protons was observed. At this point the reaction mixture was lyophilized, redissolved in acetic acid (2 mL, pH 3.5), and applied to a Sephadex G-10 column (1.5 × 85 cm), equilibrated, and eluted with the same solvent. The eluate was monitored for increased conductivity. The first major high-conductivity peak that emerged from the column was 2-deoxyribose 5-phosphate. This peak was pooled and lyophilized. Proton NMR spectra (not shown) show diminution of the two proton peaks assigned to the 2-protons.

Synthesis of 2′-Deoxyguanosine-*2′,2′-d*$_2$. A solution of guanine in 1 M HCl (7 ml, 300 μmol) was added to buffer (700 mL, 0.1 M Tric-Cl, 0.1 mM $MnCl_2$, pH 8.1). The solution was then brought back to pH 8.1 by addition of concentrated NaOH. Glucose 1,6-diphosphate (1.6 mL, 2.15 μmol) was added, followed by calf thymus purine nucleoside phosphorylase (88 units). All the 2-deoxyribose 5-phosphate-*2,2-d*$_2$ purified previously and phosphopentomutase (50 units) were added to initiate the reaction. The mixture was incubated overnight at room temperature and then lyophilized. Lyophilized material was dissolved in water (40 mL) and the pH was adjusted to 3.5 by addition of glacial acetic acid. The solution was then centrifuged and the precipitate discarded. The solution (20 mL) was applied to a sephadex G-10 column (4 × 60 cm), equilibrated, and eluted with pH 3.5 acetic acid. Eluate was monitored for absorbance at 260 nm and conductivity. Conductivity of the eluate was used to separate salt from guanosine. Appropriate fractions were pooled and lyophilized: extent of deuterium incorporation, 62.5%; yield, 11 mg (13% of theoretical).

Preparation of Thymidine-*2′,2′-d*$_2$. Thymine (1010 μmol), glucose 1,6-diphosphate (7.5 μmol), phosphopeutomutase (12 units), thymidine phosphorylase (200 units), and 2-deoxyribose 5-phosphate-*2,2-d*$_2$ (250 mg) were added to buffer (67.5 mL, 0.1 M Tris-Cl, pH 8.2, 0.1 mM $MnCl_2$). After 18 h of incubation at room temperature, the reaction mixture was lyophilized. The lyophilized material was dissolved in acetic acid (20 mL, pH 3.5) and applied to a sephadex G-10 column (4 × 60 cm), equilibrated, and eluted with pH 3.5 acetic acid. The eluate was monitored for conductivity and absorbance at 260 nm. The first major UV absorbing peak was thymidine. Appropriate fractions were pooled, lyophilized, and redissolved in water and applied to a AG50W-X8 column in H^+ form and eluted with water. Thymidine came through without appreciable retention, free of Tris. It was then lyophilized to remove the residual acetic acid. Extent of deuterium incorporation (75%); yield, 11.3 mg (5% of theoretical yield); molar extinction coefficient = 10 500.

(15) Parko, R. E.; Agarwal R. P. "The Enzymes", 3rd ed.; Boyer, P., Ed.; Academic Press: New York, 1972; Vol. VII.
(16) Keepers, J. W.; James, T. L. *J. Am. Chem. Soc.* **1982**, *104*, 929–939.
(17) Lipari, G.; Szabo, A. *Biochemistry* **1981**, *20*, 6250–6256.