from planarity. With these severe structural changes, the Me2 and H19 protons remained sufficiently close to give a weak cross peak in the back-calculated 2D NOESY spectrum (supplementary material).

We have been unable to generate a *reverse* F430 model that is simultaneously consistent with the experimental interproton distance constraints and has low primary covalency violations. Since both structures can be manipulated to be consistent with experimental data, we cannot unambiguously assign the stereochemistry of C19. However, our data indicate that the reverse structure, if it exists, should contain internal strain.

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Supplementary Material Available: Back-calculated 100-ms NOESY spectrum and cross-peak buildup curves associated with Me2 for a representative reverse model generated by inclusion of the Me2–H19 (4.8 Å–infinity) constraint as well as the buildup curves of the cross peaks and decay of the autopeaks associated with the Me2 protons for the experimental, DG "original", and DG "reverse" structures described in Figure 1 (3 pages). Ordering information is given on any current masthead page.

On the Use of Model Compounds To Assess 2-Deoxy-D-*erythro*-pentofuranose Conformation at Apyrimidinic Sites in DNA

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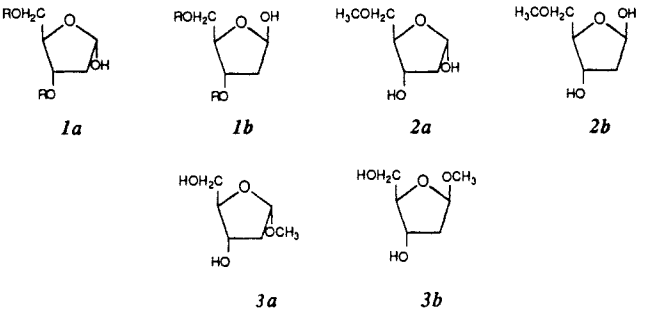
The biological mechanisms of 2′-deoxyribonucleic acid (DNA) repair in vivo are many and varied.[1] For example, it is well-known that cytosine (C) bases in DNA undergo spontaneous deamination in vivo to produce uracil (U) bases, the latter being foreign to normal DNA structure.[2] While the spontaneous conversion of C to U occurs infrequently ($k = 2 \times 10^{-10}$ s^{-1} for single-stranded DNA, $k = 1 \times 10^{-12}$ s^{-1} for double-stranded DNA),[1] this mutation threatens DNA sequence integrity and thus must be corrected efficiently. The initial repair step involves the removal of the uracil base via *N*-glycoside bond cleavage catalyzed by uracil-DNA glycosylase,[3-5] producing an apyrimidinic (AP) site in the DNA molecule. Subsequent processing by DNA polymerase and ligase excises the deoxy sugar and inserts the correct C residue.

The transient AP site is composed of 2-deoxy-α-D- and -β-D-*erythro*-pentofuranosyl rings (**1a** and **1b**, respectively) (Scheme I) linked to the DNA strand via two phosphodiester bonds. Using stable isotopically enriched DNA oligomers, Gerlt and co-workers[6,7] found that the anomeric distribution of **1** at an AP site generated in a single-stranded DNA oligomer was similar to that observed for 5-*O*-methyl-2-deoxy-D-*erythro*-pentose (**2**) (Scheme I) in aqueous solution. Recently Raap and co-workers[8] proposed that methyl 2-deoxy-α-D-*erythro*-pentofuranoside (**3a**) and methyl 2-deoxy-β-D-*erythro*-pentofuranoside (**3b**) are good conformational models of **1** in abasic DNA. A least-squares analysis[9a] of $^3J_{HH}$

Scheme I



1a *1b* *2a* *2b* *3a* *3b*

Table I. ^{1}H–^{1}H Spin-Coupling Constants[a] in 2-Deoxy-5-*O*-methyl-D-*erythro*-pentofuranoses in ^2H_2O at 25 °C

coupled nuclei	α-furanose **2a**	β-furanose **2b**
1,2	5.5 (5.4)[b]	5.1 (2.7)
1,2′	2.4 (1.3)	4.0 (5.5)
2,2′	−14.2	−14.0
2,3	7.1 (7.5)	5.7 (6.7)
2′,3	3.7 (2.5)	6.5 (5.9)
3,4	4.5 (3.6)	4.4 (4.2)
4,5	3.2	4.1
4,5′	6.0	7.2
5,5′	−11.0	−10.9

[a] Coupling constants are expressed in hertz and are accurate to ±0.1 Hz. [b] Values in parentheses are corresponding couplings reported previously for **3a** and **3b**[8] at 19 °C.

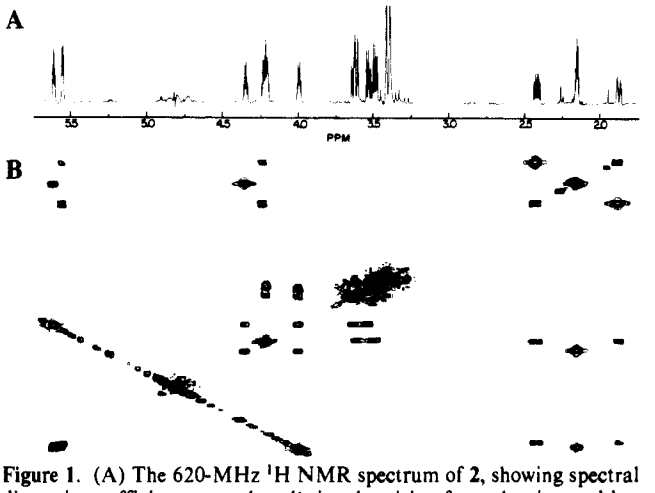


Figure 1. (A) The 620-MHz ^{1}H NMR spectrum of **2**, showing spectral dispersion sufficient to resolve all signals arising from the ring and hydroxymethyl protons of both anomers. Chemical shifts in parts per million relative to the internal HOD signal (4.80 ppm) are as follows: for **2a**, 5.50 (H1), 2.37 (H2), 1.83 (H2′), 4.17 (H3), 4.15 (H4), 3.56 (H5), 3.44 (H5′), 3.37 (CH_3); for **2b**, 5.55 (H1), 2.12 (H2), 2.09 (H2′), 4.29 (H3), 3.94 (H4), 3.58 (H5), 3.49 (H5′), 3.34 (CH_3). (B) The ^{1}H–^{1}H COSY spectrum of **2** obtained at 620 MHz used to assign specific proton multiplets to each anomer via the off-diagonal elements.

(1) For a recent review, see: Sancar, A.; Sancar, G. B. *Annu. Rev. Biochem.* **1988**, *57*, 29.
(2) The presence of uracil in DNA may also arise from misincorporation by DNA polymerase, or deamination of cytosine catalyzed by bisulfite ion or nitrous acid.[1]
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data obtained on **3b** at 19 °C[9b] (Table I) gave the following conformational parameters: $P(N) = 335°$ (E_2), $\tau_m(N) = 45°$, $P(S) = 234°$ (^4E), $\tau_m(S) = 42°$, and $X_n = 0.52$, where $P(N)$ and $P(S)$ are the phase angles of pseudorotation[10] for north (N) and south (S) conformers, respectively, $\tau_m(N)$ and $\tau_m(S)$ are the puckering amplitudes of the N and S conformers, respectively, and X_S is the mole fraction of S conformers. Thus, the preferred N and S conformers of **3b** were found to be E_2 and ^4E, respectively, and both conformers are puckered ~43° and have comparable stabilities. However, are **3a** and **3b** reasonable model structures of **1** in abasic DNA? We have addressed this question by evaluating the conformational behavior of the furanose anomers of 5-*O*-methyl-2-deoxy-D-*erythro*-pentose **2**[11] (Scheme I). In contrast to the glycosides **3a** and **3b**, **2** anomerizes spontaneously in aqueous solution to give α-furanose **2a** and β-furanose **2b** (Scheme I), thus mimicking the behavior of **1** in abasic DNA more closely than **3a** and **3b**.

In opposition to arguments made by Raap and co-workers,[8] the 620-MHz ^{1}H NMR spectrum of **2** (Figure 1A) is disperse enough to permit signal assignments to each furanose anomer with the assistance of ^{1}H-^{1}H COSY data (Figure 1B). A least-squares treatment[9a] of $^3J_{HH}$ in the predominant β-anomer **2b** (Table I) gave $P(N) = 334°$ (E_2), $P(S) = 154°$ (^2E), and $X_n = 0.58$, with $\tau_m(N)$ and $\tau_m(S) \approx 40°$. Thus, the conformational behavior of **2b** differs significantly from that of **3b** with respect to the preferred S conformer (in **3b**, ^4E is preferred, whereas in **2b**, ^2E is preferred), although the mole fractions of N and S conformers for **2b** and **3b** are similar. Computations conducted with $^3J_{HH}$ values observed in **2a** (Table I) gave $P(S)$, $\tau_m(S)$, and X_n similar to those found for **3a**,[8] but different values of $P(N)$ and $\tau_m(N)$. In **2a**, $P(N) = 0°$ and $\tau_m(N) = 43°$, whereas values of 29° and 26°, respectively, were reported for **3a** at 19°.[8]

These results show that methyl glycosidation affects the conformational behavior of the 2-deoxy-D-*erythro*-pentofuranosyl ring and that its effect is not identical in both anomers. Hence, **3b** and, to some extent, **3a** are probably not good conformational models of **1** in abasic DNA. In aqueous solution, the OCH_3 functionality at C1 of **3** appears to provide preferential stability to furanose conformers having the C1–O1 bond quasi-axial;[12] this is not unexpected, since the "anomeric effect"[13] is likely to be more pronounced in methyl furanosides than in the corresponding furanoses. These expectations are consistent with the observation that the ^4E conformer of the glycoside **3b** (its preferred S conformer) orients the C1–O1 bond near quasi-axial, whereas the ^2E conformer of the reducing sugar **2b** (its preferred S conformer) orients this bond near quasi-equatorial. In contrast, since the E_1 conformer having C1–O1 quasi-axial is already highly preferred (~80%) by **3a**, little change is expected, and is observed, upon methyl glycosidation.

Thus, conformational extrapolations based on the behavior of model compounds must be viewed with caution, especially when conformationally flexible structures such as aldofuranose rings are the focus of attention. An understanding of the conformational properties of **1** in abasic DNA, and the role these properties may play in mediating biological recognition during DNA repair, will more likely derive from direct studies of the 2-deoxy-D-*erythro*-pentofuranose anomers at AP sites in small DNA oligomers, rather than from studies of simple monosaccharide models.

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Unequivocal Demonstration of the Involvement of a Glutamate Residue as a Nucleophile in the Mechanism of a "Retaining" Glycosidase

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The exact nature of the involvement of aspartic acid and glutamic acid residues in catalysis by glycosidases has been a matter of considerable debate for some years.[1,2] Evidence has accrued for the involvement of a carboxylic acid as the acid catalyst in several glycosidases, such evidence resting primarily upon X-ray crystallographic studies,[3] as well as more recent site-directed mutagenesis experiments.[4,5] A carboxylate residue has also been implicated in stabilizing the positive charge of an oxocarbonium ion transition state or intermediate, but the question of whether it forms a covalent intermediate has not been fully answered.[1,2] The case for the stabilized ion-pair intermediate has rested upon X-ray crystallographic studies of hen egg white lysozyme,[3] where an aspartic acid residue (Asp 52) was found to be suitably positioned to stabilize such an intermediate. Labeling studies with mechanism-based inhibitors and affinity labels have shown that a carboxylate group might be similarly disposed in a number of other glycosidases.[6] In several other cases,[7-9] a slow substrate (either a D-glycal or an aryl 2-deoxyglycoside) has been used to generate a steady-state concentration of the corresponding glycosyl enzyme intermediate which was stabilized by denaturation trapping and digested with proteases and the purified, labeled peptide then sequenced. All these experiments suggest some involvement of a carboxylate side chain in stabilizing an intermediate, but as pointed out recently,[10,11] none have directly proven the involvement of a carboxyl group in a covalent linkage with a catalytically competent intermediate since the inactivator studies did not involve catalytically competent species, and since denaturation trapping does not allow distinction between covalent and ion-pair intermediates. In this paper we demonstrate that the covalent 2-deoxy-2-fluoro-α-D-glucopyranosyl enzyme intermediate generated by reaction of a β-glucosidase with the corresponding glycosyl fluoride or 2,4-dinitrophenyl glycoside[12,13] is catalytically competent since addition of a second sugar to this species promotes turnover and generation of a disaccharide glycoside product. In addition we demonstrate that a glutamate residue (Glu 358) is the amino acid through which this intermediate is attached.

We have recently[12-14] described the use of 2-deoxy-2-fluoroglucosides with reactive leaving groups (dinitrophenolate or fluoride) in trapping a covalent intermediate involved in the normal

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