VOLUME 67, NUMBER 17

JOC Articles

AUGUST 23, 2002

A Highly Effective Nonpolar Isostere of Deoxyguanosine: Synthesis, Structure, Stacking, and Base Pairing

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Received April 30, 2002

We describe the preparation and structure of the deoxyribonucleoside of 4-fluoro-6-methylbenzimidazole, abbreviated dH (**8**), which acts as a close shape mimic of the nucleoside deoxyguanosine. The nucleoside is prepared from 2-fluoro-4-methylaniline in seven steps. The X-ray crystal structure reveals a (−*sc*) glycosidic orientation, an *S* conformation for the deoxyribose moiety, and quite close shape mimicry of guanine by the substituted benzimidazole. Conformational studies by ^{1}H NMR and ^{1}H−^{1}H ROESY experiments reveal an *S*-type conformation and an anti glycosidic orientation in solution (D_2O), essentially the same as that of deoxyguanosine. Base-stacking studies in a "dangling end" context reveal that the benzimidazole base mimic stacks more strongly than all four natural bases, and more strongly than its counterpart guanine by 1.1 kcal/mol. Base-pairing studies in a 12mer DNA duplex show that, like other nonpolar nucleoside isosteres, H is destabilizing and nonselective when paired opposite natural bases. However, when paired opposite another nonpolar isostere, difluorotoluene (F), a mimic of thymine, the pair exhibits stability approaching that of its natural analogue, a G−T (wobble) base pair. The nucleoside analogue dH will be useful in studies of protein−DNA interactions, and the H−F base pair will serve as a structurally and thermodynamically close mimic of G−T in studies of DNA mismatch repair enzymes.

Introduction

Nonpolar nucleoside isosteres[1] have proven to be useful tools in probing the active sites of DNA polymerase enzymes and DNA repair enzymes.[2] These shape-mimicking molecules lack hydrogen bonding ability, and so, by comparison with their natural counterparts, act as probes of electrostatic effects in the absence of large steric differences. To date we and others have reported on properties of an isostere for thymidine (dF) in which difluorotoluene replaces the thymine nucleobase.[3] In addition, we have described the structures and properties of nonpolar isosteres for deoxyadenosine, in which 4-methylbenzimidazole (dZ)[4] or 4-methylindole[5] replaces the adenine base.

To date there has been no report of a proposed shape mimic for deoxyguanosine. Such a molecule has a shape different from that of the previous isosteres, as a result of distinct fluorine and methyl substitution. This difference is expected to be useful, since variations in shapes of nucleobases are thought to have large effects on polymerase efficiency and fidelity.[6] In addition, a shape mimic for dG would be very beneficial in probing mechanisms of DNA damage repair. The "wobble" mismatch G−T appears in DNA relatively frequently as a result of misinsertion errors by DNA polymerases, and from

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10.1021/jo025884e CCC: $22.00
Published on Web 07/09/2002

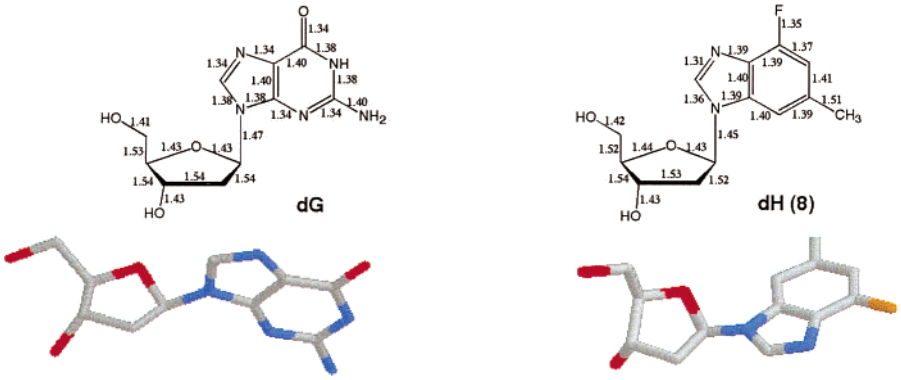
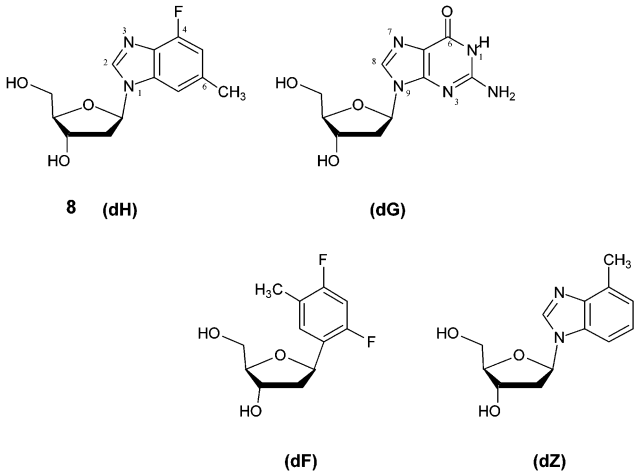


FIGURE 1. The solid-state structure of nucleoside mimic dH (**8**) compared to that of deoxyguanosine (dG). Bond lengths are given in angstroms.

deamination of 5-methylcytosine.[7] For the majority of DNA polymerases, the G−T mismatch is the most common error made. Because of the high frequency of G−T mismatches in cells, there are several known repair enzymes that recognize and correct these errors in prokaryotic and eukaryotic systems.[7]

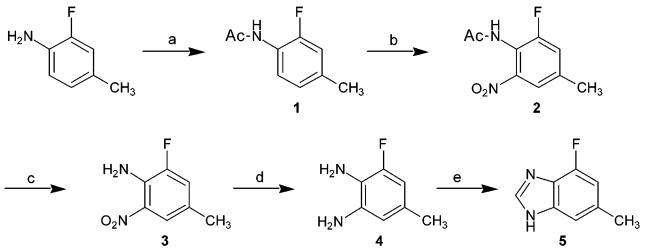


Here we describe the preparation and properties of the new isostere dH (**8**), a highly effective shape mimic for dG. We find that it stacks surprisingly strongly in DNA, and it selectively pairs with dF, resulting in a fully hydrophobic, non-H-bonded pair (H−F) that mimics the natural G−T mismatch quite closely.

Results

Synthesis. Starting with commercial 2-fluoro-4-methylaniline, the nucleobase mimic 4-fluoro-6-methyl-1*H*-benzimidazole (**5**) was synthesized in five steps as detailed in Scheme 1. Benzimidazole **5** was then coupled with 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose in the presence of sodium hydride.[8] The two resulting isomers ("fluorine up" and "fluorine down") were then deprotected with sodium methoxide and separated by column chromatography to yield 5-(4-fluoro-6-methylbenzimidazol-1-yl)-β-D-deoxyriboside **8** (abbreviated dH).

SCHEME 1[a]



[a] Conditions: (a) Ac_2O, $CHCl_3$ (94%); (b) HNO_3 (28%); (c) KOH/MeOH (86%); (d) H_2, Pd/C (95%); (e) formic acid, heat (67%).

The "fluorine up" and "fluorine down" isomers were initially distinguished by 2D $^1H-^1H$ ROESY experiments, and the faster moving isomer (by column chromatography) was assigned as the desired "fluorine up" isomer **8**.

The assignment was later confirmed by X-ray crystallography (see below). Full details of synthesis and characterization are given in the Supporting Information.

Nucleoside dH (**8**) was converted to the standard 5′-*O*- dimethoxytrityl-3′-*O*-cyanoethylphosphoramidite derivative (**10**) in two additional steps in preparation for automated DNA synthesis (Scheme 2). Incorporation into oligodeoxynucleotides was carried out with standard coupling cycles, and stepwise yields for coupling of this compound were >98% by trityl monitoring. Its incorporation into DNA was confirmed both by NMR spectroscopy of short oligonucleotides containing dH and by MALDI-TOF mass spectrometry (see Supporting Information).

Solid-State Structure. The crystal structure of dH and the known crystal structure of dG[9] are shown in Figure 1. The nucleobase structures are quite similar; the only significant deviations occur where the nitrogen-to-carbon replacements were made. This affects the bond lengths of C6−C7 (a difference of 0.05 Å) and C7−C8 (0.06 Å). The exocyclic substituents also show reasonable mimicry in bond lengths, with the C-methyl bond length in dH 0.11 Å longer than that of C−NH_2 in dG, and the C−F bond in dH within 0.01 Å of the corresponding C═O bond in dG.

Although the bond lengths and base shapes in the two structures are quite close, there are differences in the

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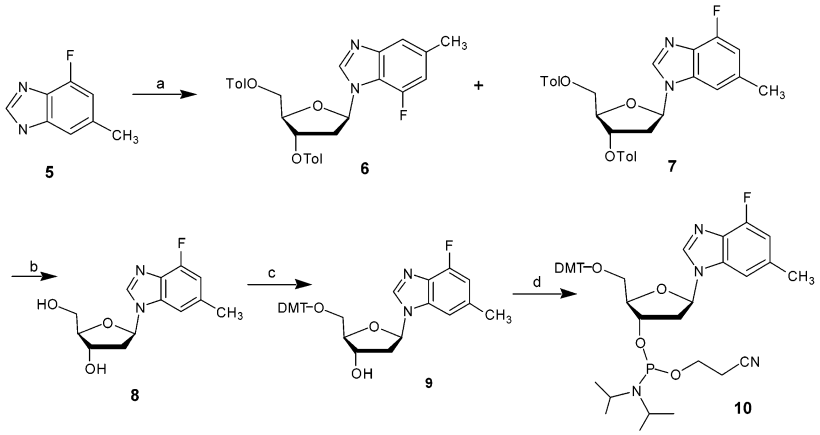
SCHEME 2[a]



[a] Conditions: (a) NaH, CH_3CN (46%, "fluorine up"); (b) NaOMe, MeOH (65%); (c) DMT-Cl, pyridine, DMAP (98%); (d) cyanoethylphosphonamidic chloride, DIPEA (80%).

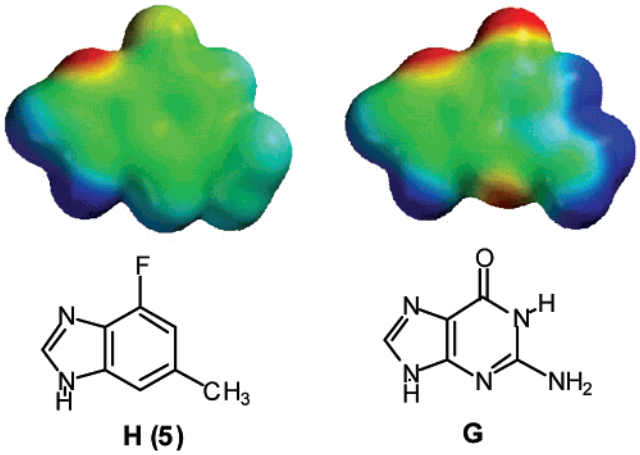


FIGURE 2. Space-filling models of the natural nucleobase guanine and the nonpolar mimic H (**5**). Electrostatic potentials are mapped on the surfaces. Red denotes negative potential and blue positive potential.

orientation of the base with respect to the furanose ring. The natural product dGadopts a typically anti conformation with a torsional angle (χ) of −122°. The base of dH is twisted by 58° relative to this, falling between typical anti and syn ranges, and displaying a −*sc* (high anti) glycosidic conformation with a measured torsional angle (χ) of −65°. The two nucleosides adopt different sugar conformations in the solid state. Analogue dH has a $C_{2'}$-endo (south) sugar pucker and dG has an $O_{4'}$-endo conformation.

Space-filling models of the nucleobase G and mimic H show very close similarity in shape (Figure 2). Once again, the single noticeable difference is at the proton at C-7 in H (compared to N3 of G). Comparison of calculated electrostatic potentials (AM1) on these molecules confirms the highly polar nature of G, and shows by contrast that H is expected to be quite nonpolar, except at the nitrogen in the imidazole ring (analogous to N7 of guanine).

Conformation in Solution. There are even greater structural similarities between the two nucleosides in D_2O solution as indicated by the nuclear magnetic resonance data (Table 1). Sugar conformations of both

TABLE 1. ^{1}H NMR Data (D_2O) for Nucleoside 8 (dH) and Deoxyguanosine

	chemical shift					
	H1′	H2′	H2″	H3′	H4′	H5′
nucleoside **8** (ppm)	6.36	2.75	2.50	4.56	4.05	3.70
deoxyguanosine	6.24	2.73	2.46	4.56	4.06	3.73

nucleosides are essentially identical, and are classified as 70% S for dG and 66% S for dH.

Relative intensities of ^{1}H−^{1}H cross-peaks, as determined by 2D ^{1}H−^{1}H ROESY experiments, were examined for both dH and dG in D_2O. While there are small differences in orientation, the data indicate that both nucleosides adopt an anti conformation about the glycosidic bond. For dH, a strong H2−H2′ nuclear Overhauser effect, a weak H2−H2″ effect, and a medium H7−H2′ effect were detected.

Stacking and Pairing Studies in DNA. The stacking properties of dH were measured in the context of duplex DNA by the dangling end method. For these preliminary studies, one standard[10] sequence context was chosen, with the dangling dH residue adjacent to C in the self-complementary duplex. Table 2 shows the data in comparison to literature values for other related compounds.[11]

The data show strong stabilization by the nucleobase mimic H in this context, with stacking considerably stronger than that of G, and close to that of the most strongly stacking molecules (pyrene, 5-nitroindole) in a recent study.[11]

Pairing of base mimic H opposite natural bases and opposite another nonpolar base mimic was evaluated in a 12 base pair sequence context. The analogue H is quite destabilizing when paired opposite natural nucleobases (Table 3), and shows little if any selectivity between them. However, when paired opposite the nonpolar thymine mimic difluorotoluene (F) it regains some stability, and the overall duplex with the H−F pair approaches

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TABLE 2. Free Energy of Stacking for Natural Nucleosides and Related Analogs, As Measured by Dangling End Thermal Denaturation Studies with Self-Complementary Strands (dXCGCGCG)[a]

dangling residue	T_m (°C)[b]	$-\Delta H°$ (kcal) (van't Hoff)	$\Delta S°$ (eu) (van't Hoff)	$-\Delta G°_{37}$ (eu) (van't Hoff)	$-\Delta G°_{37}$ (kcal) (fits)[c]	$\Delta\Delta G°$ stacking
none (core duplex)[e]	41.7	46	120	8.1 ± 0.2	8.1 ± 0.1	
thymine[e]	48.1	48	130	9.2 ± 0.2	9.2 ± 0.2	1.1 ± 0.2
adenine[e]	51.6	55	140	10.1 ± 0.2	10.0 ± 0.4	2.0 ± 0.2
cytosine[e]	46.2	50	130	9.1 ± 0.2	8.9 ± 0.1	1.0 ± 0.2
guanine[e]	51.5	43	110	9.4 ± 0.2	9.9 ± 0.3	1.3 ± 0.2
4-F-6-Me-benzimidazole (H)	55.7	70	190	11.6 ± 0.2	10.7 ± .05	3.5 ± 0.5
4-methylindole[e]	54.6	67	180	11.2 ± .2	10.5 ± .1	3.1 ± .3
5-nitroindole[e]	60.6	54	140	11.4 ± .2	11.6 ± .3	3.4 ± .3

[a] Free energy of stacking ($\Delta\Delta G°$) is obtained by substracting the free energies of the duplexes with two dangling residues from the energy of the core hexamer duplex. [b] Conditions: 1 M NaCl, 10 mM Na·phosphate, pH 7.0; 5.0 μM DNA strand concentration for T_m value shown. [c] Average free energies from fits to individual melting curves. [d] Concentration 6 μM. [e] Data from ref 11.

TABLE 3. Base Pairing Data for Guanine Analog H Opposite Natural Bases and Opposite Thymine Analog F

duplex sequence	T_m (°C)[a]	$-\Delta H°$ (kcal) (van't Hoff)	$\Delta S°$ (eu) (van't Hoff)	$-\Delta G°_{37}$ (kcal) (van't Hoff)	$-\Delta G°_{37}$ (kcal) (fits)[b]
5'AAGAAAGAAAAG[c] 3'TTCTTTCTTTTC	39.8	99	290	nd	12.4± 1.0
AAGAAHGAAAAG TTCTTACTTTTC	28.5	62	180	6.6 ± .1	6.7 ± .1
AAGAAHGAAAAG TTCTTCCTTTTC	28.2	91	280	5.6 ± .4	6.5 ± .1
AAGAAHGAAAAG TTCTTGCTTTTC	30.0	100	320	6.0 ± .9	6.9 ± .1
AAGAAHGAAAAG TTCTTTCTTTTC	27.6	120	370	4.6 ±1.3	6.6 ± .1
AAGAAHGAAAAG TTCTTFCTTTTC	33.7	59	170	7.7 ± .1	7.6 ± .1
AAGAAGGAAAAG TTCTTTCTTTTC	36.7	76	220	8.3 ± .1	8.3 ± .1

[a] Conditions: 100 mM NaCl, 10 mM Na·PIPES, pH 7.0; 10 mM $MgClL_2$, 5.0 μM DNA strand concentration for T_m. [b] Average free energies from fits to individual melting curves. [c] Data from ref 17.

that of the duplex containing the analogous G−T "wobble" pair.

Discussion

Structure Mimicry by dH. The NMR data show that the new nucleoside dH is an effective shape mimic of deoxyguanosine in solution. Both adopt the "*S*" class of sugar conformation in water, and are virtually identical in this conformation, as judged by coupling constants in the sugar protons.[12] In addition, both the natural nucleobase and the substituted benzimidazole base mimic appear to adopt predominantly anti glycosidic orientations.

It is not surprising that in the crystalline state the new nucleoside dH adopts a glycosidic orientation twisted significantly from that in the published structure of dG. Purines are known to adopt both syn and anti conformations under varying conditions, both in the crystalline form and in solution. Since rotation about the glycosidic bond is relatively unhindered in purines, a wide variety of torsional angles have been reported in the X-ray structures of deoxyguanine, deoxyguanosine, and guanosine monophosphate.[9,13] For example, a 1:1 complex of deoxyguanosine and 5-bromodeoxycytidine crystallizes with the deoxyguanosine in the syn conformation.[14] We surmise (i) that rotation about this bond is relatively unhindered for dH (as with dG) and (ii) that crystal packing effects have influenced the dH conformation. A similar effect was seen for a nonpolar isostere of deoxyadenosine in the solid state,[4] and later structural studies in the context of DNA[5] revealed it to adopt the anti orientation, like its natural counterpart dA.

The crystal structure of dH is useful in pointing out the extent of shape mimicry by the base mimic 4-fluoro-6-methylbenzimidazole. Comparison with the guanine heterocycle in the dG structure shows very similar structures. The one most substantial structural difference between dG and dH is the presence of the proton on dH at the 7 position (see Figure 2); this is missing in dG, where a ring nitrogen occurs. This difference of ca. 0.5 Å in steric bulk should be considered in enzyme active sites where close minor groove contacts might be made. In addition, the lack of a hydrogen bond acceptor at this position may have significant effects on DNA stability and on activity in DNA replication.[15]

Strong Base Stacking. The base-stacking studies show that the nucleobase mimic H is quite effective at stacking and stabilizing the double helix, at least in the one sequence context studied. Further studies in other contexts will be needed to test the generality of this. We find that H stacks considerably more strongly in this context than all four DNA bases, and surpasses guanine by a large 1.1 kcal/mol per residue. Since calculated polarizability (data not shown) and the sizes of G and H are essentially the same, we hypothesize that this difference is due to enhanced hydrophobic effects for H rather than to differences in dispersion forces. Comparison of this molecule with other nonpolar nucleoside replacements[11,16] shows that H stacks somewhat more strongly than 4-methylindole, and essentially the same as 5-nitroindole and even the tetracyclic hydrocarbon pyrene. Some of the enhanced stacking may be due to increased surface area, increased hydrophobicity of fluorine, and/or altered electrostatics. Work is underway to test some of these effects more systematically.

Base Pairing. The results show that nonpolar isostere dH pairs almost equally with all four natural bases. It is

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quite substantially destabilizing relative to a standard A−T base pair. The H−X pairs are all approximately as destabilizing as a mismatch in this context.[17] We attribute this in part to the lack of hydrogen bonds and, perhaps more importantly, to the energetic cost of desolvation of the polar partners paired across from H. Virtually the same results have been observed previously for the other nonpolar isosteres dF and dZ.[4,18] We note that nonpolar, non-hydrogen bonding nucleosides are among the most effective candidates for "universal" DNA bases that pair equally with all four bases.[19]

Interestingly and notably, the data show that pairing of dH with another nonpolar nucleoside (dF) results in greater stability for the duplex, relative to the pairs of H with polar bases. The stability of the H−F pair is ∼1 kcal/mol more stable than the H−T pair, for example. Thus the H−F pair is selective for pairing in the context of the four natural pairs. We attribute this preference of nonpolar base for another one to removal of the desolvation cost that is incurred when one partner is polar, and perhaps also to more favorable hydrophobic effects.[17]

The Watson−Crick pairing faces of dH and dF are essentially identical in shape and size to those of the natural nucleosides dG and dT. Since H−F is potentially a close shape analogue of the G−T "wobble" mismatch, it is interesting to compare H−F to G−T in pairing stability. The results here show that G−T is more stable than H−F by a relatively small amount (∼0.6 kcal). We hypothesize that the reason for the lower stability for H−F, despite the strong stacking of F and H, may by due either to a lack of hydrogen bonds or to disruption of minor groove solvation.[20]

We anticipate that analogue dH will be useful in studies of DNA replication and repair. For example, a recently discovered error-prone human polymerase, Pol iota, is reported to synthesize G−T mismatches with exceptionally high frequency.[21] The analogue H could be useful in probing steric and hydrogen bonding effects in the active sites of such error-prone enzymes. In addition, G−T mismatches, for reasons described above, occur commonly in cells. The analogue H, and the analogous H−F pair, may well be useful in probing mechanisms of repair enzymes that correct these mutagenic mismatches.[7]

Experimental Section

General Remarks. ^{1}H, ^{13}C, and ROESY NMR spectra were obtained on a 300 MHz instrument. Column chromatography was performed with Merck silica gel 60 (230−400 mesh). 2-Fluoro-4-methylaniline was purchased from a commercial source. Anhydrous dimethylformamide and acetonitrile were purchased from a commercial source in Sure-seal bottles. X-ray crystallography was performed at the Small Molecule X-ray Facility of the Department of Chemistry and Biochemistry at the University of California, San Diego.

X-ray Crystallographic Methods. Single crystals of nucleoside **8** were grown from a concentrated ethanol solution. A clear crystal of dimensions 0.20 × 0.13 × 0.10 mm^3 was mounted on a glass fiber with epoxy. The X-ray intensity data were collected at 100(2) K, at a wavelength of 0.71073, with a θ range of 1.58−27.50. Frames were integrated to yield a total of 10252 reflections, of which 2722 were independent (R_{int} = 2.98%). The crystal belongs to the orthorhombic crystal system. The unit cell parameters were a = 5.8291(6) Å, b = 7.9860(8) Å, c = 25.797(3) Å, and V = 1200.9(2) Å^3. The space group was assigned as $P2(1)2(1)2(1)$. The structure was solved with direct methods included in the SHELXTL program package and refined by full-matrix least-squares on F^2. For a Z value of 4, there is one molecule in the asymmetric unit. All non-hydrogen atoms were refined anisotropically with hydrogens included in idealized positions giving a data: parameter ratio of approximately 15:1. The largest peak in the final difference map was 0.321 e/Å^3. The structure refined to a goodness of fit (GOF) on F^2 of 0.915 and final residuals of R1 = 3.42% and wR2 = 8.60%.

***N*-(2-Fluoro-4-methylphenyl)acetamide (1).** A solution of 2-fluoro-4-methylaniline (4.90 g, 39.2 mmol) diluted in 50 mL of chloroform was stirred and cooled to 0 °C. To that solution was added dropwise acetic anhydride (4.0 g, 39.2 mmol) diluted in 100 mL of chloform over 10 minutes. The mixture was stirred for an additional 30 min. Water was added, the solution was neutralized with a saturated solution of Na_2CO_3, and the product was extracted into the organic layer. The combined organic layers were dried with magnesium sulfate and concentrated to yield 6.27 g (94%) of **1** as a white solid: ^{1}H NMR ($CDCl_3$) δ 8.11 (1H, dd, J = 8.1, 8.7 Hz), 7.31 (1H, NH), 6.91 (2H, m), 2.31 (3H, s), 2.20 (3H, s). ^{13}C NMR ($CDCl_3$) δ 168.50, 150.92, 125.13, 122.02, 115.60, 115.36, 24.72, 21.08. HRMS calcd for $C_9H_{10}FNO$ M + H m/e 168.0825, found 168.0822.

2-Fluoro-4-methyl-6-nitroaniline (3). The protected aniline derivative (**1**) (6.27 g, 37.5 mmol) was dissolved in ice cold 70% HNO_3 (70 mL). Maintaining the temperature at 0 °C, 90% HNO_3 (60 mL) was added dropwise over 1 h. The solution was stirred for one additional hour then poured over ice to form a yellow precipitate. The solid was collected by vacuum filtration, washed with water until the filtrate was neutral, and dried to yield 6.66 g (83.8%) of a mixture of nitrated product **2** (*N*-acetamido-2-fluoro-4-methyl-6-nitroaniline) and the 5-nitro isomer as a pale yellow solid. The mixture was dissolved in 100 mL of methanol and stirred. Solid KOH was added until the pH was 10. While the reaction was stirred for several hours, solid KOH was added as needed to maintain the pH at 10. The methanol was removed by evaporation under reduced pressure and the deprotected product **3** was extracted with methylene chloride. The combined organic layers were dried with magnesium sulfate. The crude product was concentrated and purified by silica column chromatography ($CHCl_3$−EtOAc, 5:1) to obtain 1.50 g (24%) of **3** as a yellow/orange oil: ^{1}H NMR (DMSO) δ 7.66 (1H, s), 7.34 (1H, d, J = 11.7 Hz), 2.22 (3H, s). ^{13}C NMR ($CDCl_3$) δ 20.40, 124.79, 124.88, 132.95, 133.19, 150.24, 153.45. HRMS calcd for $C_7H_7FN_2O_2$ M + NH_4^+ m/e 188.0835, found 188.0840.

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1-Fluoro-5-methyl-2,3-diaminobenzene (4). Approximately 25 mg of Pd(C) (5 wt %) was added to **3** (2.24 g, 13.2 mmol) dissolved in 5 mL of anhydrous DMF. The mixture was hydrogenated at 52 psi overnight. The purple-colored mixture was filtered over Celite and concentrated to yield 1.75 g (95%) of **6** as a blue/purple oil: ^{1}H NMR ($CDCl_3$) δ 6.36 (1H, d, J = 10.5 Hz), 6.31 (1H, s), 2.19 (3H, s). ^{13}C NMR ($CDCl_3$) δ 20.74, 112.16, 119.06, 129.55, 137.18, 151.46, 154.57. HRMS calcd for $C_7H_9FN_2$ M + H *m/e* 141.0828, found 141.0831.

4-Fluoro-6-methyl-1*H*-benzimidazole (5). Formic acid (1.6 mL, 88%) was added to 1.75 g (10.7 mmol) of diamine **4** dissolved in 20 mL of methanol. The mixture was heated to reflux. During the reflux the pH was maintained at approximately 7 by the addition of 88% formic acid. After approximately 48 h of reflux, the reaction mixture was cooled then concentrated to remove the methanol. Water and ethyl acetate were added, and the product was extracted into the organic layer. The combined organic layers were dried, then concentrated. The crude material (1.58 g, 10.53 mmol) was purified by silica column chromatography ($CHCl_3$:EtOAc, 3:1). Benzimidazole **5** was obtained as a pale yellow oil, 1.25 g (67%): ^{1}H NMR ($CDCl_3$) δ 8.02 (1H, s), 7.19 (1H, s), 6.82 (1H, d, J = 11.1 Hz), 2.47 (3H, s). ^{13}C NMR ($CDCl_3$) δ 155.19, 151.94, 142.71, 135.47, 110.21, 109.97, 21.80. GC/MS calcd for $C_8H_7N_2F$ M *m/e* 150.15, found 150.

1′,2′-Dideoxy-3′,5′-di-*O*-toluoyl-1′-(4-fluoro-6-methyl-1*H*-benzimidazole)-*β*-D-ribofuranose (7). Benzimidazole **5** (1.25 g, 8.33 mmol) was dissolved in dry CH_3CN (100 mL). Sodium hydride (60% suspension in mineral oil) (367 mg, 9.2 mmol) was added and the mixture was stirred in a nitrogen atmosphere for 1 h at room temperature while hydrogen gas evolved. The mixture was cooled to 0 °C, then 3.28 g of 1′-α-chloro-3′,5′-di-*O*-toluoyl-2′-deoxyribose[9] (8.33 mmol) was added in one portion. The mixture was stirred and slowly warmed back to room temperature. The reaction was stirred overnight then quenched by the addition of a saturated solution of $NaHCO_3$. The reaction mixture was filtered and concentrated, and the product was extracted with ethyl acetate. The combined organic layers were dried and concentrated to recover the mixture of isomers (**6** and **7**) as a brown oil. The isomers were separated and purified by silica column chromatography (hexane:EtOAc, 1:1). Compound **7** was recovered as a clear oil (1.93 g, 46%). ^{1}H NMR (DMSO) δ 8.53(1H, s), 7.80 (2H, d, J = 7.8 Hz), 7.83 (2H, d, J = 7.8 Hz), 7.35 (1H, s), 7.34 (4H, m), 7.01 (1H, d, J = 12.6 Hz), 6.61 (1H, dd, J = 6.9, 7.2 Hz), 5.70 (1H, m), 4.53 (3H, m), 3.09 (1H, m), 2.81 (1H, m), 2.41 (3H, s), 2.38 (3H, s), 2.84 (3H, s). ^{13}C NMR ($CDCl_3$) δ 166.06, 165.87, 155.35, 152.00, 144.53, 144.18, 140.00, 135.65, 135.53, 134.79, 134.70, 129.93, 129.74, 129.55, 129.27, 126.60, 126.37, 109.87, 109.64, 106.41, 85.48, 82.50, 74.69, 63.90, 38.06, 21.65, 21.57. HRMS calcd for $C_{29}H_{27}O_5N_2F$ M + H *m/e* 503.1982, found 503.1990.

1′,2′-Dideoxy-3′,5′dihydroxy-1′-(4-fluoro-6-methyl-1*H*-benzimidazole)-*β*-D-ribofuranose (8). To a solution of **7** bis-toluoyl ester (1.18 g, 3.85 mmol) in absolute ethanol (20 mL) was added NaOMe (in methanol, 25%, 0.5 mL). The reaction mixture was stirred for 3 h. Solid ammonium chloride was added to quench the reaction. The mixture was filtered then concentrated. Ethyl acetate and water were added and the product was extracted. The combined organic layers were dried, concentrated, and purified with use of silica column chromatography to yield 415 mg (65%) of nucleoside **8** as a clear oil. ^{1}H NMR (D_2O) δ 8.26 (1H, s), 7.25 (1H, s), 6.92 (1H, d, J = 12.3 Hz), 6.36 (1H, dd, J = 3.6, 6.6 Hz), 4.56 (1H, m), 4.05 (1H, m), 3.70 (2H, m), 2.75 (1H, m), 2.50 (1H, m), 2.41 (3H, s). ^{13}C NMR (D_2O) δ 152.97, 152.94, 121.09, 120.87, 118.31, 107.23, 97.84, 96.07, 81.92, 72.44, 49.66, 31.94, 26.68. HRMS (DCI) calcd for $C_{13}H_{15}O_3N_2F$ (M + H) 267.1145, found 267.1150.

1′,2′-Dideoxy-5′-(4,4′-dimethoxitrityl)-3′-hydroxy-1′-(4-fluoro-6-methyl-1*H*-benzimidazole)-*β*-D-ribofuranose (9). The deprotected nucleoside (**8**), 200 mg, 0.751 mmol) was coevaporated with dry pyridine (2 × 5 mL) and dissolved in pyridine (9 mL). To the above mixture was added diisopropylethylamine (315 μL) in one portion. A solution of 4,4′-dimethoxitrityl (DMT) chloride (382 mg, 1.12 mmol) in pyridine (5 mL) was added slowly over 40 min. The mixture was stirred for 22 h and quenched by adding methanol (20 mL). The mixture was concentrated and purified by flash column chromatography (hexanes−ethyl acetate−triethylamine, 1:2:0.1) to obtain 438 mg (98%) of the 5′-DMT ether derivative as a yellow foam. ^{1}H NMR ($CDCl_3$) δ 7.95 (1H, s), 7.40−7.15 (10 H, m), 6.80 (1H, d, J = 12 Hz), 6.77 (4H, d, J = 9.0 Hz), 6.25 (1H, t, J = 7.2 Hz), 4.80 (1H, m), 4.68 (1H, br s), 4.24 (1H, m), 3.77 (6H, s), 3.35 (2H, m), 2.70−2.38 (2H, m), 2.30 (3H, s); ^{13}C NMR ($CDCl_3$) δ 158.28, 154.71, 151.37, 144.34, 140.13, 135.66, 135.54, 135.46, 134.53, 134.44, 130.19, 129.96, 129.77, 127.99, 127.54, 126.59, 112.91, 109.58, 109.35, 106.89, 86.34, 86.20, 85.21, 71.42, 63.68, 60.15, 54.84, 40.22, 21.33. HRMS (DCI) calcd for $C_{34}H_{33}O_5N_2F$ (M + 1) 569.2452, found 569.2452.

1′,2′-Dideoxy-5′-(4,4′-dimethoxitrityl)-1′-(4-fluoro-6-methyl-1*H*-benzimidazole)-*β*-D-ribofuranose-3′-*O*-cyanoethyl-*N*,*N*-diisopropylphosphoramidate (10). The 5′-*O*-tritylated compound (**9**) (100 mg, 0.176 mmol) was dissolved in dry dichloromethane (2.5 mL), and to this were added diisopropylethylamine (0.170 mL, 0.66 mmol) and 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (0.08 mL, 0.35 mmol). The reaction mixture was stirred at room temperature for 1 h and 45 min. Hexanes (5 mL) was added and the concentrated mixture was purified by flash column chromatography (hexanes−ethyl acetate−triethylamine, 3:2:0.1). The two isomers of **10** were obtained as oils, DMT phosphoramidite A (52 mg, 38%), and DMT phosphoramidite B (56 mg, 42%). Spectroscopic data for DMT phosphoramidite A: ^{1}H NMR ($CDCl_3$) δ 7.98 (1H, s), 7.42−7.13 (10H, m), 6.85−6.72 (5H, m), 6.26 (1H, m), 4.81−4.70 (1H, m), 4.33 (1H, m), 3.90−3.59 (3H, m), 3.80 (6H, s), 3.43−3.25 (2H, m), 2.80−2.25 (5H, m), 2.30 (3H, s), 1.26−1.12 (12H, m). ^{13}C NMR ($CDCl_3$) δ 158.48, 144.27, 140.13, 135.72, 135.43, 134.46, 134.37, 129.96, 128.09, 127.71, 126.81, 117.16, 113.04, 109.57, 109.34, 106.63, 106.60, 86.40, 85.59, 85.55, 85.18, 73.55, 73.32, 63.16, 58.37, 58.11, 55.07, 43.31, 43.15, 39.44, 39.40, 24.49, 24.40, 21.45, 20.12, 20.03. HRMS (FAB, 3-NBA matrix) calcd for $C_{43}H_{50}O_6N_4FP$ (M + 1) 768.3530, found 768.3546. Spectroscopic data for DMT phosphoramidite B: ^{1}H NMR ($CDCl_3$) δ 7.99 (1H, s), 7.42−7.15 (10H, m), 6.85−6.72 (5H, m), 6.28 (1H, m), 4.70 (1H, m), 4.31 (1H, m), 3.90−3.52 (3H, m), 3.80 (6H, s), 3.42−3.27 (2H, m), 2.80−2.59 (5H, m), 2.33 (3H, s), 1.22−1.12 (12H, m). ^{13}C NMR ($CDCl_3$) δ 158.52, 144.30, 140.15,

135.70, 135.45, 134.50, 129.97, 128.10, 127.72, 126.80, 117.18, 113.07, 109.60, 109.32, 106.65, 106.61, 87.01, 85.80, 85.72, 85.55, 74.69, 74.47, 63.84, 58.81, 58.55, 55.29, 44.06, 43.93, 39.84, 39.81, 24.63, 24.54, 21.52, 20.50, 20.41. HRMS (FAB, 3-NBA matrix) calcd for $C_{43}H_{50}O_6N_4FP(M + 1)$ 768.3530, found 768.3501.

Acknowledgment. We thank the National Institutes of Health (GM52956) for support. D.T. acknowledges the University of San Diego Faculty Research Grants for support and Dr. Tammy Dwyer for helpful discussions.

Supporting Information Available: Proton NMR spectra for all compounds and tables of supporting data and an ORTEP diagram for the X-ray structure of compound **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO025884E