Synthesis of Oligonucleotides and Thermal Stability of Duplexes Containing the β-C-Nucleoside Analogue of Fapy·dG

Michael O. Delaney[†] and Marc M. Greenberg*[,‡]

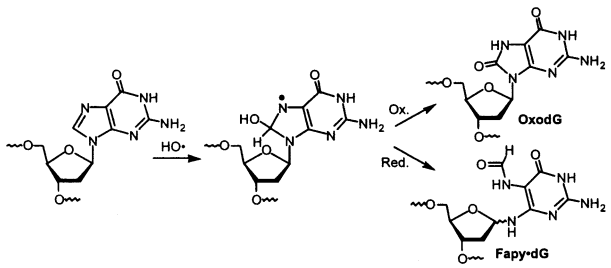
Department of Chemistry, Johns Hopkins University, Baltimore, Maryland 21218, and Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523

Received July 31, 2002

The formamidopyrimidine lesions (Fapy·dA, Fapy·dG) are formed in significant amounts when DNA is exposed to oxidative stress. These lesions are unusual in that they readily epimerize in solution. The distribution of configurational isomers in DNA is unknown. Nonepimerizable, nonhydrolyzable analogues are useful probes for investigating the configuration of Fapy lesions in DNA and as potential enzyme inhibitors. The β-C-nucleoside of Fapy·dG has been prepared and introduced sight-specifically into oligonucleotides via its respective β-cyanoethyl phosphoramidite. The phosphoramidite was prepared via a Wittig reaction between a protected form of deoxyribose and a suitably functionalized pyrimidine. The pyrimidine contained methyl and 2-propyl groups at the O4 and O2 positions, respectively, to differentiate between them following C-nucleoside formation. The formamide was derived from a nitro group at C5. The phosphoramidite coupled in 80% yield via a single 15-min coupling using tetrazole as activator. Oligonucleotides as long as 36 nucleotides were prepared and characterized by ESI-MS.

DNA damage is involved in aging and a variety of diseases, such as cancer (*1*, *2*). Nucleobase modification is a general form of DNA damage resulting from initial alkylation, reduction, or oxidation (*3*, *4*). Deoxyguanosine is the most readily oxidized of the four nucleotides that make up DNA, and a great deal of effort has gone into determining the effects of lesions such as OxodG on polymerases and repair enzymes (*5*−*7*). Fapy·dG is formally the same oxidation state of deoxyguanosine, but it is formed via an initial oxidative event (e.g., hydroxyl radical addition). The formamidopyrimidines and 8-oxopurines are produced via a common intermediate (Scheme 1) (*8*). 8-Oxopurine formation is favored under oxygen-rich conditions, but the formamidopyrimidines are favored under anoxic conditions and as a result of UV irradiation (*9*, *10*). Furthermore, there is at least one instance in which Fapy·dG formation is favored over OxodG in cells. The Fapy·dG:OxodG ratio is almost 3 in human leukemia cells (*11*). The biological importance of formamidopyrimidine lesions is also indicated by their efficient removal by base excision repair (BER) enzymes found in a variety of species, including humans (*5*, *12*−*15*). Despite the apparent importance of formamidopyrimidine lesions, much less is known about them than the respective 8-oxopurines. This is largely due to the absence of a method for synthesizing oligonucleotides containing formamidopyrimidines until recently. Incorporation of lesions and their analogues at defined sites in oligonucleotides facilitates determining their effects on DNA structure and function. Recently, we described methods for synthesizing oligonucleotides containing

Scheme 1



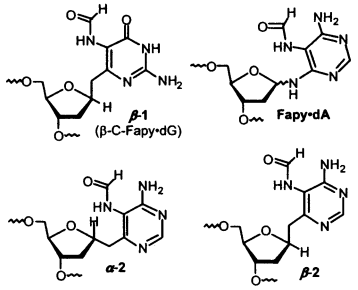
Fapy·dG, Fapy·dA, and the latter lesion's C-nucleoside analogues (*16*, *17*). Herein we describe the synthesis and characterization of oligonucleotides containing β-C-Fapy·dG (β-**1**).

Nonhydrolyzable analogues of nucleosides are useful tools for probing the interactions of DNA with repair enzymes (*18*−*20*). Unlike most lesions, including the chemically related 8-oxopurines, Fapy·dG and Fapy·dA epimerize rapidly in aqueous solution (*21*, *22*). The ratio of configurational isomers in DNA is unknown, but the β-anomer of each monomeric species is favored slightly at room temperature. Configurationally stable analogues of the formamidopyrimidines are particularly valuable, because they may also provide insight regarding which anomer(s) is (are) responsible for the impact on DNA−protein interactions. Recently, the interactions of DNA containing Fapy·dA or its individual anomeric C-nucleoside analogues with Klenow exo$^-$ and formamidopyrimidine DNA glycosylase (Fpg, MutM) were analyzed. The kinetics of translesional synthesis and binding by Fpg of DNA containing β-**2** closely mimicked Fapy·dA, whereas the behavior of α-**2** did not correlate with that of the true lesion (*23*, *24*). In fact, β-**2** is a nanomolar inhibitor (K_I = 7.5 ± 1.3 nM) of Fpg excision of Fapy·dA. Based upon

* Address correspondence to this author. E-mail: mgreenberg@jhu.edu.
[†] Colorado State University.
[‡] Johns Hopkins University.

10.1021/tx025588x CCC: $22.00
Published on Web 10/17/2002

these observations, it was proposed that the *β*-anomer of the C-nucleoside closely represents the structure of Fapy·dA. In addition, the correlation between the behavior of *β*-**2** and Fapy·dA suggested that the glycosidic nitrogen in the lesion is not involved in specific interactions with the polymerase and repair enzyme examined. More recently, we reported that Fapy·dG induces Klenow exo$^-$ to misincorporate dA opposite it and extend the prior 80 million times more efficiently than does the native purine (*25*). Because Fapy·dG also epimerizes readily and we anticipated that a nonhydrolyzable analogue of this lesion would exhibit interesting inhibition properties, using the above observations regarding Fapy·dA as a guide, we chose to prepare oligonucleotides containing *β*-C-Fapy·dG (*β*-**1**).



Experimental Section

General Methods. ^{1}H NMR spectra were recorded at 300 or 400 MHz. The NMR spectra are referenced according to the solvent ($CDCl_3$, 7.27 ppm, or CD_3OD, 3.31 ppm). ^{13}C NMR were recorded at 75 or 100 MHz and referenced according to the solvent ($CDCl_3$, 77.23 ppm; CD_3OD, 49.15 ppm). ^{31}P NMR spectra were obtained at 109 MHz and referenced according to H_3PO_4 as an external standard (H_3PO_4 = 0 ppm). IR spectra were obtained using an Avatar 320-FT-IR spectrophotometer. The Central Instrument Facility at Colorado State University performed LRMS and HRMS FAB. ESI-MS was performed on a Thermoquest LCQ-DUO. Oligonucleotide melting studies were performed in 1 cm path length quartz cells on a Beckman DU 640 UV−Vis spectrophotometer equipped with a thermoprogrammer.

Oligonucleotide synthesis was carried out on an Applied Biosystems 394 DNA/RNA synthesizer using standard protocols. *N*-Acetyl-protected deoxycytidine-*β*-cyanoethyl phosphoramidite was purchased from Pharmacia Biotech. Phenoxyacetyl-protected deoxyadenosine, isopropylphenoxyacetyl-protected deoxyguanosine, and other standard phosphoramidites were purchased from Glen Research. All other oligonucleotide synthesis reagents were also obtained from Glen Research. Gel electrophoresis on 20% polyacrylamide denaturing gels (PAGE) afforded the purified oligonucleotide. Oligonucleotides were precipitated from NH_4OAc/EtOH prior to analysis by ESI-MS.

Preparation of 8. A solution of **7** (*30*, *33*) (14.59 g, 73.3 mmol) and Ag_2O (32.60 g, 139.3 mmol) in 2-propanol (240 mL) was heated to reflux. After 24 h, the solution was filtered through a pad of Celite and concentrated. The resulting oil was dissolved in EtOAc (100 mL) and washed with saturated $NaHCO_3$ (50 mL). The aqueous layer was extracted with EtOAc (3 × 50 mL), and the combined organics were concentrated in vacuo. The crude material was purified by column chromatography (100% hexanes to 2% EtOAc) to give the product (**8**) as a white solid (7.06 g, 42%) and **7** (2.63 g, 18%). ^{1}H NMR ($CDCl_3$) δ 5.27 (h, 1 H, J = 6.0 Hz), 4.01 (s, 3 H), 2.42 (s, 3 H), 1.34 (d, J = 6.4 Hz, 6 H); ^{13}C NMR ($CDCl_3$) δ 163.79, 163.42, 162.48, 129.57, 72.11, 55.30, 21.76, 21.19; mp 82−84 °C; IR (film) 2982, 1581, 1519, 1428, 1378, 1316, 1113, 794 cm^{-1}; HRMS (M+H) for $C_9H_{14}N_3O_3$: calcd 228.0984, found 228.0985.

Preparation of 10. To a solution of **8** (5.13 g, 22.6 mmol) and NaOAc (3.70 g, 45.2 mmol) in AcOH (100 mL) was added a 9:1 (v/v) solution of AcOH and Br_2 (4.0 mL, 7.76 mmol). The solution was heated to reflux until the red color dissipated. The reaction was cooled to room temperature, and additional solution (1:9, v/v) of Br_2 in AcOH was added (4.0 mL, 7.76 mmol), and the reaction was returned to reflux. After 30 min, another portion of Br_2 solution was added (5.0 mL, 9.70 mmol) in a similar manner, and the reaction was refluxed for 30 min and concentrated in vacuo. The resulting oil was purified by column chromatography (100% hexanes to 2% EtOAc) to give the monobromide product (**9**) that was contaminated with residual starting material (3.32 g, 48%) and dibromide (3.91 g, 45%). **9**: ^{1}H NMR ($CDCl_3$) δ 6.79 (s, 1 H), 5.40 (h, 1 H, J = 6.4 Hz), 4.10 (s, 3 H), 1.45 (d, 6 H, J = 6.4 Hz); ^{13}C NMR ($CDCl_3$) δ 164.04, 163.50, 159.91, 124.18, 73.66, 56.31, 33.67, 21.78; mp 83−84 °C; IR (film) 2982, 1577, 1523, 1485, 1427, 1386, 1324, 1105 cm^{-1}. The mixture of **8** and **9** (3.32 g, 10.85 mmol) was dissolved in benzene (20 mL), and PPh_3 (3.98 g, 15.2 mmol) in benzene (16 mL) was added dropwise. The reaction was stirred for 4.5 h and filtered. The resulting white salt was washed with benzene (2 × 30 mL). The phosphonium bromide was dissolved in a biphasic mixture of $CHCl_3$ (70 mL) and ice/water (40 mL). To this mixture is added 3 M NaOH (3.65 mL, 11.0 mmol), and the reaction was stirred vigorously. After 1.5 h, the aqueous layer was extracted with $CHCl_3$ (2 × 30 mL), and the combined organic layers were washed with brine. The solution was concentrated to leave **10** (4.19 g, 79% or 38% from **8**) as an orange foam. ^{1}H NMR ($CDCl_3$) δ 7.68−7.45 (m, 15 H), 5.21 (d, 1 H, J = 26.4 Hz), 3.99 (s, 3 H), 3.81(h, 1 H, J = 6.0 Hz), 0.66 (d, 6 H, J = 6.3 Hz); ^{13}C NMR ($CDCl_3$) δ 166.64, 161.64, 161.58, 159.40, 132.92, 132.79, 132.38, 132.01, 131.88, 129.05, 128.90, 128.49, 128.32, 126.76, 125.55, 116.85, 68.80, 57.16, 55.64, 54.61, 21.62; ^{31}P NMR ($CDCl_3$) δ 16.66; mp 221−223 °C; IR (film) 3059, 2976, 1560, 1519, 1435, 1394, 1310, 1256, 1114, 715 cm^{-1}; HRMS (M+H) for $C_{27}H_{27}N_3O_4P$: calcd 488.1739, found 488.1736.

Preparation of 11. A solution of 3′,5′-bis-*O*-*tert*-butyldimethylsilyloxy-2′-deoxyribose (*28*) (4.48 g, 12.35 mmol) and **10** (6.02 g, 12.35 mmol) in toluene (41 mL) was heated to reflux for 4.5 days. The solution was concentrated in vacuo and suspended in MeOH (62 mL). The solution was stirred for 1 h with NaOMe (0.2 g, 2.17 mmol). The reaction was filtered and quenched by the addition of excess NH_4Cl. The solution was concentrated in vacuo and chromatographed (elution with 2% EtOAc in hexanes to 10% EtOAc) to give a mixture of anomers of **11** as an oil (6.11 g, 87%). ^{1}H NMR ($CDCl_3$) δ 5.36−5.28 (m, 1 H), 4.64−4.57 (m, 1 H), 4.36−4.31 (m, 1 H), 4.06 and 4.05 (each as s, 3 H), 3.89−3.86 and 3.81−3.77 (each as m, 1H), 3.63−3.57 (m, 1 H), 3.52−3.42 (m, 1 H), 3.38−3.33 (m, 1 H), 3.11−3.04 (m, 1 H), 2.91−2.76 (m, 1 H), 2.29−2.22 and 1.95−1.88 (each as m, 1 H), 1.78−1.67 (m, 1 H), 1.42 (d, J = 6.3 Hz, 6 H), 0.91, 0.90, and 0.87 (each as s, 9H), 0.09, 0.08, 0.07, 0.06, 0.03, and 0.02 (each as s, 6 H); ^{13}C NMR ($CDCl_3$) δ 164.22, 164.00, 163.58, 163.38, 163.16, 162.78, 162.67, 130.56, 130.47, 88.20, 77.93, 74.39, 73.94, 72.48, 72.30, 72.15, 72.10, 72.01, 69.84, 64.70, 63.92, 63.76, 62.66, 55.45, 55.38, 44.10, 40.52, 39.70, 39.24, 26.17, 25.98, 25.87, 21.96, 21.89, 18.55, 18.17, 18.12, 14.36, −3.36, −4.52, −4.58, −4.61, −5.22, −5.27, −5.32, −5.44; IR (film) 2952, 2931, 1584, 1529, 1428, 1381, 1255, 1117, 838 cm^{-1}; HRMS (M+H) for $C_{26}H_{50}N_3O_7Si_2$: calcd 572.3187, found 572.3183.

Preparation of 12. To a solution of **11** (5.27 g, 9.22) in CH_3CN (46 mL) was added NaI (2.07 g, 13.83 mmol) and TBDMSCl (2.08 g, 13.83 mmol). The reaction was stirred at ambient temperature for 2 days and diluted with EtOAc (70 mL). The product was washed with saturated $NaHSO_3$ (30 mL), and the aqueous phase was extracted with EtOAc (2 × 30 mL). The combined organic phase was washed with brine and concentrated. The crude product was purified by column chromatography (elution with 100% DCM to 2% MeOH) to give **12** as a white foam (3.43 g, 67%) and recovered starting material (**11**, 1.42 g, 27%). ^{1}H NMR ($CDCl_3$) δ 5.41−5.33 (m, 1 H), 4.63−4.56

(m, 1 H), 4.37−4.31 (m, 1 H), 3.92−3.88 and 3.82−3.76 (each as m, 1 H), 3.63−3.57 (m, 1 H), 3.50−3.32 (m, 1 H), 3.04−2.97 (m, 1 H), 2.79−2.67 (m, 1 H), 2.29−2.24 and 1.94−1.88 (each as m, 1 H), 1.77−1.68 (m, 1 H), 1.39 (m, 6 H), 0.91, 0.90, 0.89, and 0.87 (each as s, 9 H), 0.10, 0.08, 0.07, 0.06, 0.04, and 0.03 (each as s, 6 H); ^{13}C NMR ($CDCl_3$) δ 163.98, 163.47, 163.38, 162.48, 130.68, 130,41, 87.96, 87.03, 77.54,76.90, 76.72, 74.23, 73.77, 72.06, 71.86, 63.72, 63.59, 55.21, 55.16, 40.93, 40.33, 39.49, 25.98, 25.82, 21.79, 18.35, 17.98, −4.69, −4.76, −4.80, −5.38, −5.46; IR (film) 2951, 2926, 2851, 1579, 1425, 1324, 1111, 839 cm^{-1}; HRMS (M+H) for $C_{25}H_{48}N_3O_7Si_2$: calcd 558.3031, found 558.3015.

Preparation of 13. A solution of **12** (3.43 g, 6.16 mmol) and NH_4F (2.30 g, 61.6 mmol) in MeOH (41 mL) was heated at reflux for 18 h. The solution was concentrated, and the product was purified by column chromatography (elution with 2% MeOH in CH_2Cl_2 to 8% MeOH) to give **13** as a white foam (1.62 g, 80%). ^{1}H NMR (CD_3OD) δ 5.27−5.19 (m, 1 H), 4.56−4.44 (m, 1 H), 4.24−4.18 (m, 1 H), 3.81−3.72 (m, 1 H), 3.61−3.46 (m, 2 H), 3.14−3.08 and 2.91−2.86 (each as m, 1 H), 2.86−2.71 (m, 1 H), 2.38−2.29 and 2.00−1.93 (each as m, 1 H), 1.87−1.71 (m, 1 H), 1.33 (d, J = 6.0 Hz, 6 H); ^{13}C NMR (CD_3OD) δ 167.37, 166.97, 163.45, 161.78, 161.70, 135.00, 134.60, 88.67, 86.91, 78.35, 78.14, 73.90, 73.91, 71.73, 71.59, 63.92, 63.28, 41.70, 41.21, 40.75, 40.41, 22.38; IR (film) 3361, 3174, 3071, 2922, 2849, 1687, 1568, 1308, 1100, 836 cm^{-1}; HRMS (M+H) for $C_{13}H_{20}N_3O_7$: calcd 330.1301, found 330.1300.

Preparation of 14. Diol **13** (1.50 g, 4.56 mmol) in 18 mL of MeOH was saturated with NH_3 for 20 min at 0 °C. The solution was then sealed and heated to 90 °C for 43 h. Concentration of the solution afforded the crude aminated diol (1.42 g) as a mixture with unreacted isopropyl ether (**13**). The crude material was azeotropically dried with pyridine (3 × 5 mL) and silylated with TBDMSCl (1.72 g, 11.4 mmol) and imidazole (1.37 g, 4.4 mmol) in DMF (23 mL) for 24 h. The reaction was concentrated in vacuo, and the crude oil was partitioned between EtOAc (40 mL) and water (20 mL). The aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organics were washed successively with water (15 mL), saturated $NaHCO_3$ (15 mL), and brine (15 mL). The organics were concentrated and purified by column chromatography (elution with 100% CH_2CL_2 to 2% MeOH) to give **14** as a foam (1.42 g, 61%) and **12** (0.72 g, 28%). ^{1}H NMR ($CDCl_3$) δ 4.80−4.72 and 4.78−4.62 (each as m, 1 H), 4.41−4.37 and 4.31−4.30 (each as m, 1 H), 3.96−3.94 and 3.85−3.82 (each as m, 1 H), 3.70−3.64 (m, 1 H), 3.48−3.44 (m, 1 H), 3.17−3.07 (m, 1 H), 2.94−2.90 (m, 1 H), 2.39−2.34 and 2.01−1.96 (each as m, 1 H), 1.82−1.72 (m, 1 H), 0.91, 0.90, 0.88, and 0.86 (each as s, 9H), 0.09, 0.08, 0.05, 0.04, 0.02, and 0.00 (each as s, 6 H); ^{13}C NMR ($CDCl_3$) δ 170.66, 169.57, 157.84, 154.80, 154.65, 128.48, 87.92, 86.34, 77.38, 76.27, 73.93, 72.68, 63.90, 62.57, 41.50, 40.91, 26.20, 26.13, 26.09, 26.05, 26.00, 18.49, 18.17, 18.09, −4.14, −4.22, −4.42, 4.51, −4.56, −4.67, −5.27, −5.31; IR (thin film) 3327, 3214, 3152, 2929, 2855, 1683, 1591, 1486, 1254, 1106, 834 cm^{-1}; HRMS (M+H) for $C_{22}H_{43}N_4O_6Si_2$: calcd 515.2721, found 515.2715.

Preparation of Isopropylphenoxyacetamide-Protected 14. To a solution of **14** (0.48 g, 0.93 mmol), PyBOP (0.730 g, 1.40 mmol), and isopropylphenoxyacetic acid (0.27 g, 1.40 mmol) in CH_2Cl_2 (9.3 mL) was added diisopropylethylamine (0.35 g, 2.78 mmol). The reaction was stirred at ambient temperature for 6 h and partitioned between CH_2Cl_2 (50 mL) and saturated $NaHCO_3$ (20 mL). The aqueous layer was extracted with DCM (2 × 25 mL) and washed with brine (25 mL). The solution was concentrated and purified by column chromatography (elution with 100% DCM to 2% MeOH) to give the amide as a white foam (0.46 g, 72%). ^{1}H NMR ($CDCl_3$) δ 9.35 (br s, 1 H), 7.22 (d, 2 H, J = 8.0 Hz), 6.92 (d, J = 8.0 Hz, 2 H), 4.70 (s, 2 H), 4.58−4.52 (m, 1H), 4.38−4.32 (m, 1 H), 3.92−3.89 and 3.82−3.77 (each as m, 1 H), 3.61−3.56 (m, 2 H), 3.47−3.44 and 3.40−3.35 (each as m, 1 H), 2.93−2.85 (m, 1 H), 2.81−2.77 and 2.68−2.65 (each as m, 1 H), 2.29−2.24 and 1.95−1.90 (each as m, 1 H), 1.77−1.70 (m, 1 H), 2.21 (d, J = 2.4 Hz, 6 H), 1.25 (s, 9 H), 1.23 (s, 9 H), 0.09, 0.08, 0.07, and 0.06 (each as s, 6 H), 0.05, 0.04, and 0.03 (each as s, 6 H); ^{13}C NMR ($CDCl_3$) δ 170.93, 161.39, 161.04, 154.52, 153.38, 148.59, 148.44, 144.05, 136.29, 128.06, 114.93, 88.30, 87.41, 76.10, 74.32, 73.86, 67.32, 63.88, 63.99, 41.15, 40.66, 40.55, 40.00, 33.52, 26.11, 25.98, 24.27, 18.51, 18.19, 18.11, −4.27, −4.51, −4.55, −4.64, −5.22, −5.31; IR (film) 3182, 2953, 1852, 1693, 1563, 1513, 1250, 1092, 837 cm^{-1}; HRMS (M+H) for $C_{33}H_{55}N_4O_8Si_2$: calcd 691.3559, found 691.3566.

Preparation of 15. To a solution of the above nitro amide (0.46 g, 1.07 mmol) in 3:1 MeOH:THF (13.4 mL) was added 10% Pd/C (230 mg). The suspension was charged with H_2 (45 psi) and allowed to stir for 1 h. The reaction was filtered through a pad of Celite and concentrated in vacuo. The crude amine was dissolved in 13.4 mL of THF and treated with acetic formic anhydride (0.18 g, 2.01 mmol) and pyridine (0.21 g, 2.70 mmol) at 0 °C. After 1.25 h, the solvent was removed and the material purified by column chromatography (elution with 0.1% MeOH in CH_2Cl_2 to 5% MeOH) to afford **15** as a white foam (0.37 g, 80%). ^{1}H NMR (CD_3OD) δ 8.24 (s, 1 H), 7.16 (d, J = 8.8 Hz, 2 H), 6.93 (d, J = 8.8 Hz, 2 H), 4.74 (s, 2 H), 4.53−4.49 (m, 1 H), 4.45−4.40 and 4.38−4.35 (each as m, 1 H), 3.96−3.92 and 3.84−3.80 (each as m, 1 H), 3.68−3.64 and 3.54−3.50 (each as m, 1 H), 4.62−3.59 (m, 1 H), 3.02−2.99 and 2.90−2.84 (each as m, 1 H), 2.88−2.82 (m, 1 H), 2.72−2.68 (m, 1 H), 2.38−2.34 and 1.94−1.90 (each as m, 1 H), 1.83−1.71 (m, 1 H), 1.21 (d, J = 6.8 Hz, 6 H), 0.90 (s, 9 H), 0.89 (s, 9 H), 0.09 and 0.06 (each as s, 6 H), 0.05 and 0.03 (each as s, 6 H); ^{13} C NMR (CD_3OD) δ 173.24, 163.18, 163.09, 157.21, 143.86, 128.59, 115.94, 88.33, 78.47, 77.85, 75.49, 74.83, 68.46, 64.90, 64.39, 42.28, 41.71, 34.71, 26.73, 26.62, 26.59, 26,51, 24.78, 19.32, 18.99, −4.32, −4.41, −4.96, −5.03, −5.11; IR (film) 3189, 2953, 2853, 1673, 1594, 1507, 1249, 1096, 838 cm^{-1}; HRMS (M+H) for $C_{34}H_{57}N_4O_7Si_2$: calcd 689.3766, found 689.3778.

Preparation of 16. To a solution of **15** (0.37 g, 0.54 mmol) in THF (10.6 mL) was added $Et_3N{\cdot}3HF$ (0.85 g, 5.40 mmol). The reaction was stirred for 24 h and concentrated to dryness. The crude material was purified by column chromatography (elution with 1% MeOH in EtOAc to 8% MeOH) to produce **16** as a white foam (0.21 g, 84%). ^{1}H NMR (CD_3OD) δ 8.27 and 8.25 (each as s, 1 H), 7.15−7.12 (m, 2 H), 6.93−6.69 (m, 2 H), 4.74 (s, 2 H), 4.51−4.46 (m, 1 H), 4.24−4.21 (m, 1 H), 3.93−3.90 and 3.82−3.78 (each as m, 1 H), 3.63−3.54 (m, 2 H), 2.84−2.76 (m, 2 H), 2.39−2.35 and 1.87−1.80 (each as m, 1 H), 2.04−1.98 (m, 1 H), 1.76−1.70 and 1.62−1.55 (each as m, 1 H), 1.19 (d, J = 6.8 Hz, 6 H); ^{13}C NMR (CD_3OD) d 173.32, 173.27, 167.97, 163.29, 163.20, 157.06, 150.03, 143.78, 128.54, 128.38, 117.45, 115.88, 115.57, 89.05, 87.40, 77.80, 77.70, 73.61, 73.34, 68.36, 66.22, 65.49, 63.74, 63.59, 63.41, 59.90, 52.70, 41.87, 41.76, 41.26, 34.59, 31.83, 24.73, 20.21; IR (film) 3476, 3068, 1694, 1477, 1277, 1167, 1043 cm^{-1}; HRMS (M+H) for C_{22} $H_{29}N_4O_7$: calcd 461.2036, found 461.2033.

Preparation of α,*β*-17. Diol **16** (0.10 g, 0.23 mmol) was coevaporated with pyridine (2 × 1 mL). The material was dissolved in pyridine (1.5 mL) and cooled to 0 °C. DMAP (3 mg, 0.03 mmol) and dimethoxytrityl chloride (0.11 g, 0.34 mmol) were added at 0 °C in pyridine (1 mL). The reaction was stirred for 2 h at 0 °C and allowed to warm to room temperature. The reaction was stirred an additional 1 h, concentrated, and purified by column chromatography (elution with 70% EtOAc in hexanes to 2% MeOH in EtOAc) to give α,*β*-**17** (0.13 g, 73%) as a 3:1 (*β*:α) mixture of anomers. Resolution of anomers by TLC was accomplished with 10:0.25:4 EtOAc/1-propanol/water (organic phase). The separation of anomers was facilitated by Chromatotron (1 mm plate, 100% EtOAc) to elute 93 mg of *β*-**17** first, followed by 32 mg of α-**17**. α-**17**: ^{1}H NMR (CD_3OD) δ 8.32 and 8.26 (each as s, 1 H), 7.44−7.40 (m, 2 H), 7.31−7.09 (m, 9 H), 6.84−6.70 (m, 6 H), 4.68−4.63 (m, 2 H), 4.60−4.52 (m, 1 H), 4.35−4.31 (m, 1 H), 4.11−4.04 (m, 1 H), 3.77−3.72 (m, 6 H), 3.10−3.07 (m, 1 H), 2.86−2.79 (m, 2 H), 2.45−2.38 (m, 1 H), 1.76−1.70 (m, 1 H), 1.65−1.57 (m, 1 H), 1.19 (d, J = 7.2 Hz, 6 H); ^{13}C NMR (CD_3OD) δ 173.25, 163.21, 160.21, 157.15, 146.72, 143.93, 137.52, 131.43, 129.52, 128.87, 128.60, 127.92, 115.97,

114.19, 87.52, 86.85, 78.24, 74.25, 74.12, 68.49, 65.61, 65.58, 55.83, 41.68, 34.74, 31.94, 24.75, 20.30; IR (film) 3175, 2957, 1660, 1603, 1512, 1250, 1172, 1028, 832 cm^{-1}; HRMS (M+H) for $C_{43}H_{47}N_4O_9$: calcd 763.3343, found 763.3341. β-**17**: ^1H NMR (CD_3OD) d 8.25 and 8.14 (each as s, 1 H), 7.43−7.37 (m, 2 H), 7.30−7.13 (m, 9 H), 6.91−6.80 (m, 6 H), 4.69−4.64 (m, 2 H), 4.56−4.51 (m, 1 H), 4.26−4.23 (m, 1 H), 3.98−3.96 (m, 1 H), 3.72 (s, 6 H), 3.17−3.14 (m, 1 H), 3.09−2.97 (m, 1 H), 2.88−2.77 (m, 2 H), 1.97−1.93 (m, 1 H), 1.85−1.74 (m, 1 H), 1.66−1.56 (m, 1 H), 1.20 (d, J = 9.6 Hz, 6 H); ^{13}C NMR (1:1 $CDCl_3$:CD_3OD) δ 172.10, 166.48, 162.01, 159.32, 156.08, 145.70, 143.69, 136.79, 130.87, 129.36, 128.90, 128.47, 127.48, 115.44, 113.79, 87.45, 87.01, 77.18, 67.96, 65.07, 64.76, 59.66, 55.67, 41.46, 34.09, 24.56, 14.04, 8.48; IR (film) 3201, 2957, 1655, 1611, 1577, 1511, 1250, 1037, 832 cm^{-1}. HRMS (M+H) for $C_{43}H_{47}N_4O_9$: calcd 763.3343, found 763.3349.

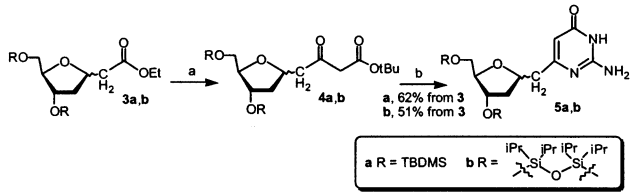
Preparation of β-6. A solution of β-**17** (47 mg, 0.06 mmol) was coevaporated with CH_3CN (4 × 1 mL). The oil was taken up in CH_2Cl_2 (1.5 mL) with diisopropylamine (6.5 mg, 0.06 mmol), and tetrazole (4.3 mg, 0.06 mmol) was added. This was followed by addition of 2-cyanoethyl tetraisopropylphosphorodiamidite (24 mg, 0.08 mmol) to the reaction. The reaction was stirred at room temperature for 5 h, and diisopropylamine (1.4 mg, 0.014 mmol), tetrazole (0.9 mg, 0.013 mmol), and 2-cyanoethyl tetraisopropylphosphorodiamidite (3.7 mg, 0.012 mmol) were added. The reaction was allowed to stir for 12 h and quenched with MeOH (1 mL). The reaction was concentrated and chromatographed on oven-dried EM-silica gel (50% EtOAc, in hexanes with 0.1% Et_3N to 0.5% MeOH in EtOAc with 0.1% Et_3N) to give β-**6** (28.2 mg, 48%). ^1H NMR (CD_3OD) δ 8.27, 8.17, and 8.26 (each as s, 1 H), 7.44−7.38 (m, 2 H), 7.32−7.12 (m, 9H), 6.93−6.81 (m, 6 H), 4.71−4.66 (m, 2 H), 4.56−4.52 (m, 1 H), 4.46−4.42 (m, 1 H), 4.11−4.04 (m, 1 H), 3.75−3.59 (m, 9 H), 3.22−3.13 (m, 3 H), 2.90−2.80 (m, 3 H), 2.67 (t, J = 5.7 Hz, 1 H), 2.54 (t, J = 5.7 Hz, 1 H), 1.94−1.87 (m, 1 H), 1.64−1.58 (m, 1 H), 1.23−1.09 (m, 18 H); ^{31}P NMR ($CDCl_3$) δ 148.41, 148.34; HRMS (M+H) for $C_{52}H_{64}N_6O_{10}P$: calcd 963.4422, found 963.4414.

Automated Synthesis of Oligonucleotides Containing β-1. With the exception of previously reported changes to the capping procedure, standard (Applied Biosystems 394) 1 μmol scale cycles were employed for coupling phosphoramidites of native nucleotides. The cycles were modified for the coupling of β-**6** so as to contain a 15-min wait time following transfer of the phosphoramidite and tetrazole solutions to the reaction column. Following coupling of β-**6**, *N*-methylimidazole was removed from the capping solution.

Oligonucleotide **18** was deprotected by treatment with 0.05 M K_2CO_3 in MeOH (2 mL) for 3 h. Deprotection of **19** and **20** under the same conditions required 5 h for complete deprotection. The deprotections were quenched with 2 equiv of AcOH and concentrated to dryness. Oligonucleotides were purified by PAGE, eluted via the crush and soak method using 0.2 M NaCl, 1 mM EDTA, and desalted on a Waters C-18 Sep-pak cartridge.

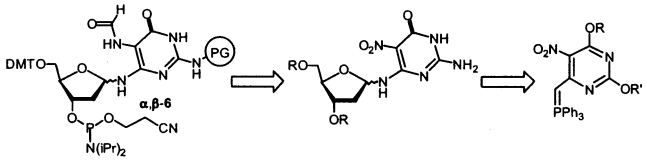
DNA Melting Experiments. The samples for the UV-melting studies consisted of a total concentration of 1.0−7.5 μM of a 1:1 molar ratio of complementary oligonucleotides. The samples were prepared by the addition of appropriate amounts of complementary oligonucleotide stock solutions to 200 μL of PIPES buffer (20 mM PIPES, pH 7.0, 20 mM $MgCl_2$, 200 mM NaCl), followed by dilution to 400 μL with water. The complementary oligonucleotides were hybridized at 90 °C for 5 min and allowed to cool to room-temperature overnight. The absorbance of the samples was then monitored at 260 nm while the temperature was increased at a rate of 0.5 °C/min over a range of 60 °C (25−85 °C). Melting temperatures were calculated by computer fit of the first derivative of absorbance with respect to T^{-1}. Thermodynamic parameters were obtained through van't Hoff analysis of the data (*34*).

Scheme 2[a]

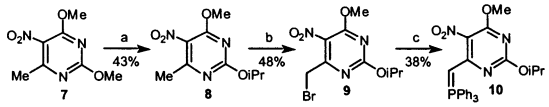


[a] Key: (a) LDA + tBuOAc, then **3**; (b) guanidine-HCl, Na_2CO_3, EtOH.

Scheme 3



Scheme 4[a]



[a] Key: (a) PrOH, Ag_2O; (b) Br_2, NaOAc, AcOH; (c) i, PPh_3, benzene, ii, $CHCl_3$, H_2O, NaOH.

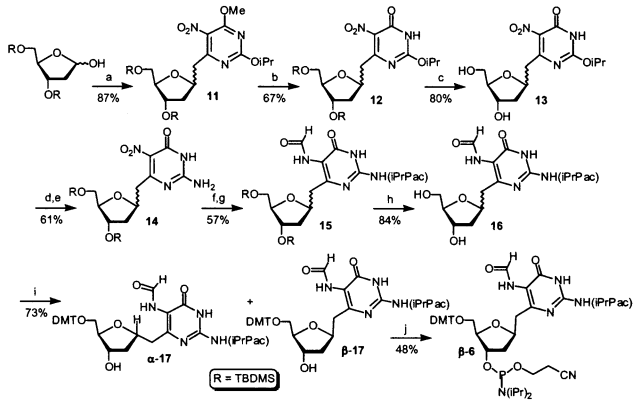
Results and Discussion

Phosphoramidite (β-6) Synthesis. Initial approaches for preparing **6** involved constructing the 2-amino-4-hydroxypyrimidine ring after forming the bond between the penultimate C-glycosidic linkage and the deoxyribose ring. The bis-TBDMS (**5a**) and 1,1,3,3-tetraisopropyldisiloxane (**5b**) nucleoside analogues were formed based upon reported procedures for similar compounds (Scheme 2) (*26*−*28*). However, we were unable to introduce a nitrogen atom at C5 of the pyrimidine (**5a**,**b**) using a variety of nitration methods, or aryldiazonium reagents. Attempts at forming the pyrimidine ring with a formamide surrogate (e.g., azide) in place also failed (data not shown).

Consequently, we explored the possibility of modifying our syntheses of α,β-**6** in which we had already solved the problem of preparing a C-glycoside pyrimidine containing a C5-nitrogen substituent (*17*). Retrosynthetic analysis led us to a 2,4-dialkoxy-6-methylene-5-nitropyrimidine ylide in which the alkoxy groups were different from one another as key intermediate (Scheme 3) (*29*, *30*). The alkoxy groups needed to be chemically distinguishable in order to differentiate between them later in the synthesis. The more hindered isopropyl group was introduced at the 2-position in order to selectively demethylate the O4-methyl group following C-glycoside formation (*31*). The Wittig ylide was prepared from **7** in a manner similar to that employed for the synthesis of the respective ylide utilized for the preparation of the α,β-**2** (Scheme 4) (*17*). Bromination was difficult to control, producing separable mixtures of mono- and dibromopyrimidines. Monobromide **9** was contaminated by small amounts of unreacted **8**, which was separated from the ylide via extraction.

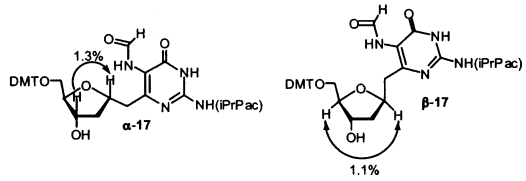
An inseparable mixture of diastereomers of **11** was obtained upon reaction of the Wittig ylide with the bis-silyl ether of deoxyribose (Scheme 5). Based upon the previous synthesis of the C-Fapy·dA compounds, we anticipated that the diastereomers would be separated

Scheme 5[a]



[a] Key: (a) i, **10**, toluene, D, ii, cat. NaOMe, MeOH; (b) TBDMSCl, NaI, CH_3CN; (c)NH_4F, MeOH; (d) NH_3, MeOH, 90 °C; (e) TBDMSCl, imidazole, DMF; (f) PyBOP, 2-(4-isopropylphenyloxy-)acetic acid, Hünigs base; (g) i, H_2, Pd/C, 3:1 MeOH THF; ii, acetic formic anhydride pyridine, THF; (h) $Et_3N{\cdot}3HF$, THF; (i) DMTCl, cat. DMAP, pyridine; (j) 2-cyanoethyl tetrapropylphosphoramidite, diisopropylamine, tetrazole, CH_2Cl_2.

Scheme 6



immediately prior to phosphitylation (*17*). Selective demethylation of **11** using in situ generated trialkylsilyl iodide proved more difficult than anticipated. Extensive desilylation of the deoxyribose protecting groups and poor selectivity for the O4-methyl group were observed when TMSI was used at 0 °C. These problems were overcome by switching to TBDMSI, which masked any attack on the deoxyribose silyl ethers, and eliminated competing dealkylation of the O2-isopropyl group. The bulkier reagent slowed the overall rate of the reaction significantly, but **12** was obtained in 67% yield along with recovered starting material (27%). Substitution of the secondary alkoxide in **12** by ammonia required vigorous conditions resulting in a mixture of products attributable to unselective desilylation of the deoxyribose. No improvement was observed upon experimentation with other nitrogen nucleophiles (e.g., hydrazine). Although additional steps were introduced, displacement of the alkoxy group was successfully performed on the desilylated material. The crude material obtained from the ammonolysis was resilylated and the desired 2-amino product (**14**) was purified by flash chromatography (61%), along with resilylated starting material (**12**, 28%). Based upon the syntheses of related molecules, transformation of the diastereomeric mixture of nitro amine (**14**) into the dimethoxytritylated C-nucleoside (**17**) was straightforward. The isopropylphenoxyacetyl protecting group was employed to facilitate chromatography of the polar-substituted pyrimidine.

Separation of the epimers of **17** required using a Chromatotron centrifugal thin-layer chromatograph following flash chromatography. Structural assignments were made by ^{1}H NMR (Scheme 6). Designation of the α-epimer was based upon observation of a NOE at H1′ when H3′ was irradiated. The expected NOE between H1′ and H4′ was not observed in the other, presumably β-epimer. However, ROSEY analysis of this diastereomer was consistent with its assignment as the β-isomer. Phosphitylation of β-**17** by the tetrazolide of 2-cyanoethyl tetraisopropylphosphorodiamidite generated in situ proceeded in moderate yield. This transformation was also only moderately effective in the Fapy·dA C-nucleoside series for reasons unknown to us (*17*). Despite significant experimentation, attempts at preparing the α-C-Fapy·dG phosphoramidite were unsuccessful. Substrate decomposition was observed under forcing conditions (extended reaction times, excess phosphitylation reagent); otherwise no reaction occurred. The reluctance of α-**17** to undergo phosphitylation as suggested by molecular modeling studies on the free nucleoside (data not shown) is attributed to steric hindrance of the 3′-hydroxyl group by the pyrimidine ring, which is tucked under the deoxyribose ring. The isopropylphenoxyacetamide in α-**17** increases congestion in the vicinity of the 3′-hydroxyl group further.

Table 1. Comparison of UV-Melting Thermodynamics of Duplexes Containing β-1, dG, or Fapy·dG[a]

5'-d(TGC AGT XAC AGC)
3'-d(ACG TCA YTG TCG)

X = β-**1**, Fapy·dG or G
Y = A, C, G, or T

X:Y	T_m (°C)[b]	$\Delta H°$ (kcal/mol)	$\Delta S°$ (e.u.)	$\Delta G°_{298}$ (kcal/mol)
β-**1**:C	46.1	70.9	194.4	13.0
β-**1**:A	40.0	51.5	137.3	10.6
β-**1**:G	37.7	51.3	136.8	10.5
β-**1**:T	39.8	39.9	101.5	9.7
Fapy·dG:C[c]	54.1	77.7	210.2	15.1
Fapy·dG:A[c]	51.7	80.1	219.0	14.8
Fapy·dG:G[c]	45.1	79.3	221.8	13.2
Fapy·dG:T[c]	44.5	68.3	187.8	12.3
G:C[c]	57.1	106.1	294.4	18.4
G:A[c]	45.5	83.0	233.7	13.3
G:G[c]	44.6	58.3	156.4	11.7
G:T[c]	46.7	83.2	232.8	13.9

[a] Conditions: PIPES (pH 7.0), 10 mM; $MgCl_2$, 10 mM; NaCl, 100 mM. [b] [Duplex] = 2.2 μM. [c] Data taken from ref *25*.

Oligonucleotide Synthesis. Incorporation of α,β-C-Fapy·dA into oligonucleotides was compromised to varying degrees by modest coupling, and transacylation in longer products (=30 nucleotides) (*17*). Employing the more commonly used activator tetrazole allowed us to use a single extended (15 min) coupling procedure to achieve 80% coupling of β-**6**. Furthermore, substituting pivalic anhydride in place of acetic anhydride during the capping procedure prevented transacylation (*32*). It is important to note that *N*-methylimidazole is not used during capping following coupling of the β-C-Fapy·dG phosphoramidite. Using these modifications, oligonucleotides 12 (**18**) and 36 (**19**, **20**) nucleotides long were prepared, purified by denaturing PAGE, and characterized by ESI-MS.

5'- d(TGC AGT XAC AGC)
18
5'- d(AGG CGT TCA ACG TGC AGT XAC AGC ACG TCC CAT GGT)
19
5'- d(AGG CGT TCA ACG TGC AGT XTC AGC ACG TCC CAT GGT)
20
X = β-**1**

Effect of β-C-Fapy·dG on DNA Melting Thermodynamics. UV-melting studies of a dodecameric duplex

containing *β*-C-Fapy·dG in an otherwise identical sequence as those previously studied containing Fapy·dG or dG were carried out (*25*). Duplexes containing the C-nucleoside analogue consistently melted at lower temperature than those containing Fapy·dG. We speculate that this is due to the conformational differences arising from substitution of a methylene group for a trivalent nitrogen. The duplex containing *β*-C-Fapy·dG opposite dC was 2.4 kcal/mol more stable than any duplex containing a mismatch, indicating that the C-nucleoside analogue was capable of selectively forming a base pair with the native nucleotide. The stability of duplexes containing *β*-C-Fapy·dG opposite mismatches also paralleled the effects observed in studies involving Fapy·dG. The duplex containing *β*-C-Fapy·dG opposite thymidine was the least stable, and those containing dA or dG opposite the C-nucleoside were destabilized to a more moderate extent (Table 1). These data are consistent with molecular modeling studies (data not shown) and indicate that *β*-**1** will be a good mimic of Fapy·dG.

Acknowledgment. We are grateful for support from the NIH (CA-74954). We thank Dr. Chris Rithner (C.S.U.) for assistance with NMR experiments.

Supporting Information Available: ESI-MS of **18**−**20**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Hoeijmakers, J. H. J. (2001) Genome maintenance mechanisms for preventing cancer. *Nature* **411**, 366−374.
(2) DePinho, R. A. (2000) The age of cancer. *Nature* **408**, 248−254.
(3) Burrows, C. J., and Muller, J. G. (1998) Oxidative nucleobase modifications leading to strand scission. **98**, 1109−1152.
(4) Gates, K. S. (1999) Covalent modification of DNA by natural products. In *Comprehensive Natural Products Chemistry, Volume 7, DNA and Aspects of Molecular Biology*, pp 491−552, Pergamon, New York.
(5) David, S. S., and Williams, S. D. (1998) Chemistry of glycosylases and endonucleases involved in base-excision repair. *Chem. Rev.* **98**, 1221−1261.
(6) Shibutani, S., Takeshita, M., and Grollman, A. P. (1991) Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-OxodG. *Nature* **349**, 431−434.
(7) Lowe, L. G., and Guengerich, F. P. (1996) Steady-state and pre-steady-state kinetic analysis of dNTP insertion opposite 8-oxo-7,8-dihydroguanine by *Escherichia coli* polymerase I exo$^-$ and II exo$^-$. *Biochemistry* **35**, 9840−9849.
(8) Candeias, L. P., and Steenken, S. (2000) Reaction of HO· with guanine derivatives in aqueous solution: formation of two different redox-active OH-adduct radicals and their unimolecular transformation reactions. Properties of G(−H)·. *Chem.*—*Eur. J.* **6**, 475−484.
(9) Doetsch, P. W., Zastawny, T. H., Martin, A. M., and Dizdaroglu, M. (1995) Monomeric base damage products from adenine, guanine, and thymine induced by exposure of DNA to ultraviolet radiation. *Biochemistry* **34**, 737−742.
(10) Gajewski, E., Rao, G., Nackerdien, Z., and Dizdaroglu, M. (1990) Modification of DNA bases in mammalian chromatin by radiation-generated free radicals. *Biochemistry* **29**, 7876−7882.
(11) Pouget, J.-P., Douki, T., Richard, M.-J., and Cadet, J. (2000) DNA damage induced in cells by *γ* and UVA radiation as measured by HPLC/GC-MS and HPLC-EC and comet assay. *Chem. Res. Toxicol.* **13**, 541−549.
(12) Dizdaroglu, M., Bauche, C., Rodriguez, H., and Laval, J. (2000) Novel substrates of *Escherichia coli* Nth protein and its kinetics for excision of modified bases from DNA damaged by free radicals. *Biochemistry* **39**, 5586−5592.
(13) Dherin, C., Radicella, J. P., Dizdaroglu, M., and Boiteux, S. (1999) Excision of oxidatively damaged DNA bases by the human α-hOgg1 protein and the polymorphic α-hOgg1(Ser326Cys) protein which is frequently found in human populations. *Nucleic Acids Res.* **27**, 4001−4007.
(14) Audebert, M., Radicella, J. P., and Dizdaroglu, M. (2000) Effect of single mutations in the *OGG1* gene found in human tumors on the substrate specificity of the Ogg1 protein. *Nucleic Acids Res.* **28**, 2672−2678.
(15) Karahalil, B., Girard, P.-M., Boiteux, S., and Dizdaroglu, M. (1998) Substrate specificity of the Ogg1 protein of *Saccharomyces cerevisiae*: Excision of guanine lesions produced in DNA by ionizing radiation- or hydrogen peroxide/metal ion-generated free radicals. *Nucleic Acids Res.* **26**, 1228−1232.
(16) Haraguchi, K., and Greenberg, M. M. (2001) Synthesis of oligonucleotides containing Fapy·dG (*N*-(2-deoxy-α,*β*-D-*erythro*-pentofuranosyl)-2,6-diamino-4-hydroxy-5-formamidopyrimidine). *J. Am. Chem. Soc.* **123**, 8636−8637.
(17) Haraguchi, K., Delaney, M. O., Wiederholt, C. J., Sambandam, A., Hantosi, Z., and Greenberg, M. M. (2002) Synthesis and characterization of oligodeoxynucleotides containing formamidopyrimidine lesions and nonhydrolyzable analogues. *J. Am. Chem. Soc.* **124**, 3263−3269.
(18) Johnson, F., Dormán, G., Rieger, R. A., Marumoto, R., Iden, C. R., and Bonala, R. (1998) Synthesis of enzymatically noncleavable carbocyclic nucleosides for DNA-*N*-glycosylase studies. *Chem. Res. Toxicol.* **11**, 193−202.
(19) Zharkov, D. O., Rosenquist, T. A., Gerchman, S. E., and Grollman, A. P. (2000) Substrate specificity and reaction mechanism of murine 8-oxoguannine-DNA glycosylase. *J. Biol. Chem.* **275**, 28607−28617.
(20) Bulycchev, N. V., Varaprasad, C. V., Dorman, G., Miller, J. H., Eisenberg, M., Grollman, A. P., and Johnson, F. (1996) Substrate specificity of *Escherichia coli* MutY protein. *Biochemistry* **35**, 13147−13156.
(21) Burgdorf, L. T., and Carell, T. (2002) Synthesis, stability, and conformation of the formamidopyrimidine G DNA lesion. *Chem.*—*Eur. J.* **8**, 293−301.
(22) Greenberg, M. M., Hantosi, Z., Wiederholt, C. J., and Rithner, C. D. (2001) Studies on *N*4-(2-deoxy-D-pentofuranosyl)-4,6-diamino-5-formamidopyrimidine (Fapy·dA) and *N*6-(2-deoxy-D-pentofuranosyl)- 6-diamino-5-formamido-4-hydroxypyrimidine (Fapy·dG). *Biochemistry* **40**, 15856−15861.
(23) Delaney, M. O., Wiederholt, C. J., and Greenberg, M. M. (2002) Fapy·dA induces nucleotide misincorporation translesionally by a DNA polymerase. *Angew. Chem., Int. Ed. Engl.* **41**, 771−773.
(24) Wiederholt, C. J., Delaney, M. O., and Greenberg, M. M., submitted for publication.
(25) Wiederholt, C. J., and Greenberg, M. M. (2002) Fapy·dG instructs Klenow exo$^-$ to misincorporate deoxyadenosine. *J. Am. Chem. Soc.* **124**, 7278−7279.
(26) Secrist, J. A., III (1978) Homo-*C*-Nucleosides. The synthesis of certain 6-substituted 4-pyrimidinones. *J. Org. Chem.* **43**, 2925−2927.
(27) Walker, J. A., Chen, J. J., Wise, D. S., and Townsend, L. B. (1996) A facile, multigram synthesis of ribofuranoid glycals. *J. Org. Chem.* **61**, 2219−2221.
(28) Wichai, U., and Woski, S. A. (1998) An improved route to 1,2-dideoxy-*β*-1-phenyl-D-ribofuranose. *Bioorg. Med. Chem. Lett.* **8**, 3465−3568.
(29) Katagiri, N., Takashima, K., Kato, S., and Tamura, C. (1983) Sythesis of homo-*C*-nucleosides using pyrimidinylmethylenephosphoranes. *J. Chem. Soc., Perkin Trans. 1*, 201−209.
(30) Cupps, T. L., Wise, D. S., Jr., and Townsend, L. B. (1986) A novel three-step synthesis of a pyrrolo[3,2-*d*]pyrimidine C-nucleoside. *J. Org. Chem.* **51**, 1058−1064.
(31) Brown, D. J., and Sugimoto, T. (1970) Aza-analogues of pteridine. Part II. The novel use of silver oxide in transetherification of alkoxy-1,2,4,6,8-penta-azanaphthalenes, alkoxy-nitropyrimidines, and related systems. *J. Chem. Soc. C*, 2661−2666.
(32) Zhu, Q., Delaney, M. O., and Greenberg, M. M. (2001) Observation and elimination of *N*-acetylation of oligonucleotides prepared using fast-deprotecting phosphoramidites and ultra-mild deprotection. *Bioorg. Med. Chem. Lett.* **11**, 1105−1107.
(33) Cupps, T. L., Wise, D. S., and Townsend, L. B. (1983) Synthetic strategies toward the synthesis of 2,4-dimethoxypyrrolo[3,2-*d*]-pyrimidine. *J. Org. Chem.* **48**, 1060−1064.
(34) Breslauer, K. J. (1995) Extracting thermodynamic data from equilibrium melting curves for oligonucleotide order−disorder transitions. *Methods Enzymol.* **259**, 221−242.

TX025588X