Synthesis of 1,N^6-Ethano-2′-deoxyadenosine, a Metabolic Product of 1,3-Bis(2-chloroethyl)nitrosourea, and Its Incorporation into Oligomeric DNA

H. Maruenda,† A. Chenna, L.-K. Liem,‡ and B. Singer*

Donner Laboratory, Lawrence Berkeley National Laboratory, University of California, Berkeley, California 94720

Received January 30, 1998

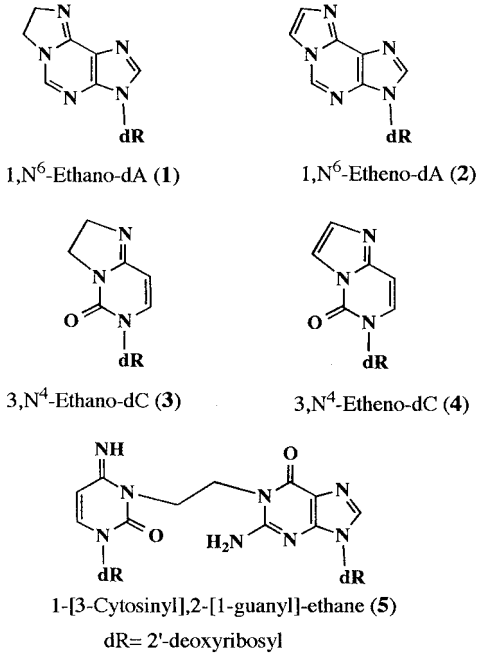
1,N^6-Ethano-2′-deoxyadenosine (**1**) is one of the adducts formed during DNA reaction with the antitumor agent, 1,3-bis(2-chloroethyl)nitrosourea (BCNU), and was synthesized and incorporated into a site-specific deoxyoligonucleotide for the first time. The product 6-chloropurine-2′-deoxyriboside (**11**) was prepared in high yield by the reaction of 2′-deoxyinosine (**6**) with $SOCl_2$, which then was derivatized to give compound **12** using *tert*-butyldimethylsilyl chloride, which was then reacted with 2-hydroxyethylamine to produce compound **13** in 86% yield. Reaction of **13** with $(PhO)_3P^+MeI^-$ in DMF gave the cyclized 1,N^6-ethano derivative **10** in 67% yield. Desilylation of **10** with triethylamine trihydrofluoride in THF gave 1,N^6-ethano-dA (**1**) in 91% yield. Tritylation of compound **1** with $DMT^+BF_4^-$ gave the 5′-*O*-DMT product **14** in 62% yield, which then was phosphitylated with 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite, which yielded a 1:1 mixture of the diastereoisomers **15** in 71% yield. This fully protected compound **15** was incorporated site-specifically into a 25-mer oligonucleotide. The coupling efficiency of ethano-dA phosphoramidite was 93%. Enzymatic hydrolysis and analysis by HPLC confirmed the incorporation of ethano-dA and base composition of the DNA oligomer. The latter is now under investigation for its biochemical and physical properties.

Introduction

1,3-Bis(2-chloroethyl)nitrosourea (BCNU) is one of a family of therapeutic nitrosoureas compounds used in cancer treatment. Reaction with DNA leads to several adducts, including saturated exocyclic adducts of adenine, cytosine, and guanine (1,N^6-ethano-A; 3,N^4-ethano-C; N^2,3-ethano-G; and 1,O^6-ethano-G),[1] which resemble the exocyclic etheno adducts formed from the reaction of the chemical carcinogen, vinyl chloride, with DNA.[2] In addition, a cross-linked DNA is formed by BCNU between the N-1 of G and the N-3 of C (1-(3-cytosinyl)-2-(1-guanyl)ethane),[3] which is believed to be the chemostatic event.[4]

All of the possible cyclic etheno derivatives have been synthesized (1,N^6-etheno-dA; 3,N^4-etheno-dC; 1,N^2-etheno-dG; and N^2,3-etheno-dG) and studied for their biochemical effects in defined oligonucleotides (reviewed by Leonard[5]). Of the possible cyclic ethano compounds, all have been characterized from the reaction of DNA with BCNU.[1] However, the first synthesis of an ethano deoxynucleoside was only recently described by Zhang et al., who synthesized and incorporated 3,N^4-ethano-dC (**3**) (Chart 1) into a site-specific oligonucleotide,[6] which enabled them to investigate how this type of adduct affects replication. They reported that in vitro replication

Chart 1



* To whom correspondence should be addressed. Tel.: (510) 642-0637. Fax: (510) 486-6488.

† Present address: Pontificia Universidad Catolica del Peru. Seccion Quimica. Apartado Postal 1761 Lima 100 Peru. E-mail: hmaruen@pucp.edu.pe.

‡ Present address: Clinical Research Center, Singapore General Hospital, Outram Road, Singapore 169608.

(1) Ludlum, D. B. *Mutat. Res.* **1990**, *233*, 117−126.

(2) Singer, B.; Holbrook, S. R.; Fraenkel-Conrat, H.; Kusmierek, J. T. In *The Role of Cyclic Nucleic Acid Adducts in Carcinogenesis and Mutagenesis*; Singer, B., Bartsch, H., Eds.; IARC Scientific Publication, Oxford University Press: New York, 1986; pp 45−58.

(3) Ludlum, D. B. In *The Role of Cyclic Nucleic Acid Adducts in Carcinogenesis and Mutagenesis*; Singer, B., Bartsch, H., Eds.; IARC Scientific Publication, Oxford University Press: New York, 1986; pp 137−146.

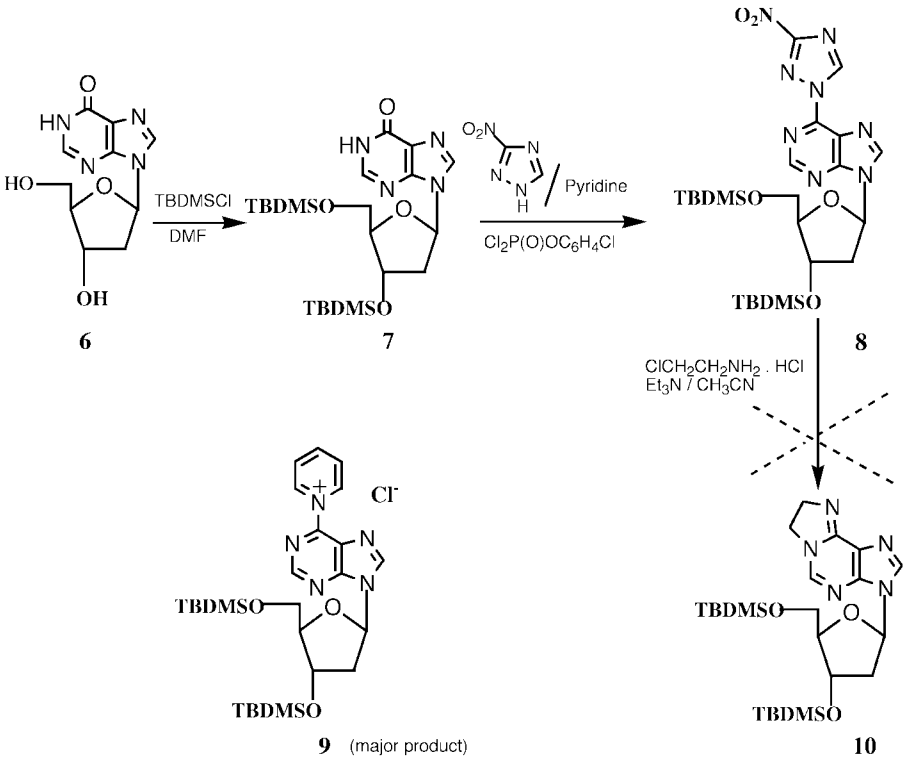
(4) Buckley, N.; Brent, T. P. *J. Am. Chem. Soc.* **1988**, *110*, 7520−7529.

(5) Leonard, N. J. *Chemtracts−Biochem. Mol. Biol.* **1992**, *3*, 273−297.

(6) Zhang, W.; Rieger, R.; Iden, C.; Johnson, F. *Chem. Res. Toxicol.* **1995**, *8*, 148−156.

S0022-3263(98)00170-4 CCC: $15.00
Published on Web 06/11/1998

Scheme 1



6 **7** **8** **9** (major product) **10**

was completely blocked, indicating this adduct would be lethal.[7] However, no synthetic route has been described for the synthesis of 1,N^6-ethano-dA (**1**) and its incorporation into a site-specific oligonucleotide.

In this paper, we report for the first time the large-scale synthesis of 1,N^6-ethano-dA (**1**), its conversion to the phosphoramidite **15** (Scheme 2), and site-specific incorporation into a 25-mer deoxyoligonucleotide. This oligonucleotide is currently being studied for its possible mutagenesis and enzymatic repair as well as physical properties.

Results and Discussion

To study the biochemical role of the 1,N^6-ethano-2′-deoxyadenosine (**1**) adduct, it was necessary to synthesize, on a large scale and in high yield, compound **1** (Scheme 1), to use as a precursor for the preparation of the corresponding phosphoramidite. This was successfully incorporated in a satisfactory yield, site-specifically in a defined 25-mer oligonucleotide.

Synthesis of 1,N^6-Ethano-2′-deoxyadenosine (1). Ludlum's laboratory has isolated and identified several adducts resulting from the reaction of BCNU with nucleosides and DNA.[8] Among them is 1,N^6-ethano-dA. However, the yields and the quantities obtained by this approach were very low. Therefore, a total synthetic method with high yield was necessary for biological and biophysical studies.

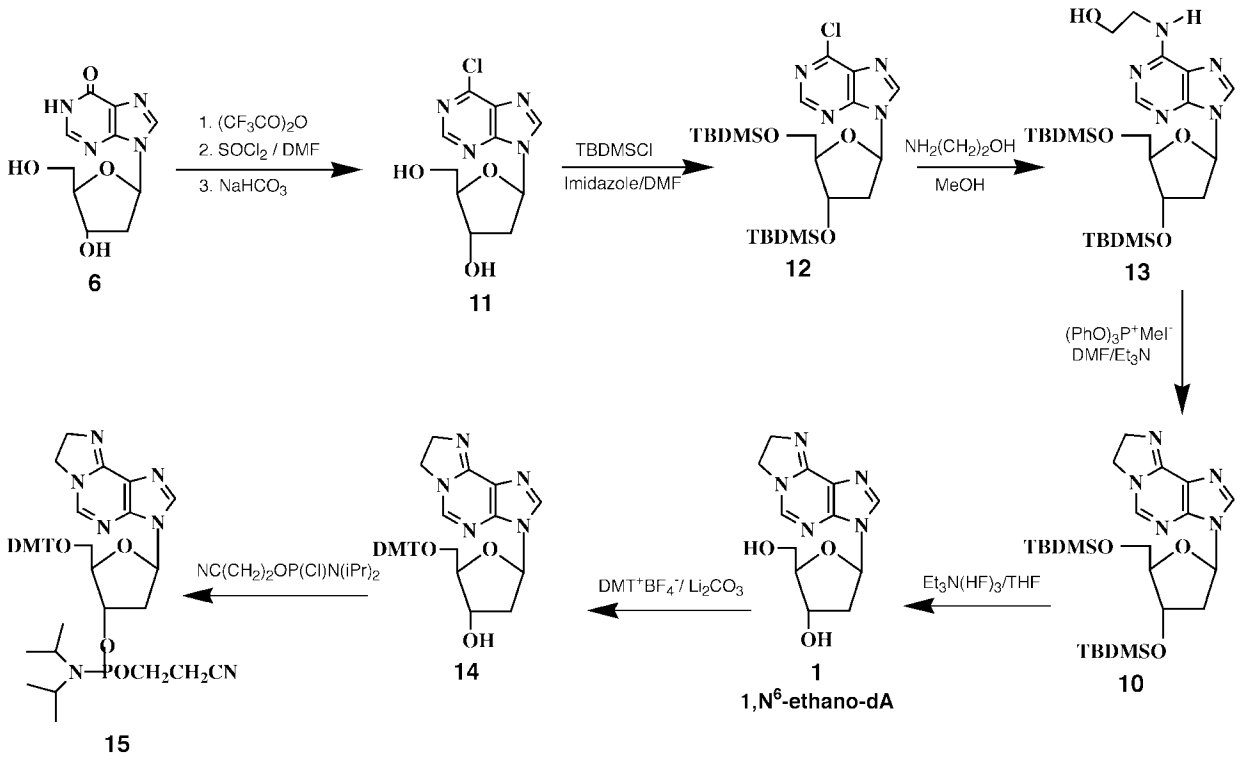
Initially, we attempted the synthesis of this compound by modifying Tong and Ludlum's[9] procedure in which 3′,5′-*O*-bis(*tert*-butyldimethylsilyl)-2-deoxyadenosine was heated with 1,2-dibromoethane in DMSO in the presence of anhydrous potassium carbonate. However, only a very low yield of the desired product (1−2%) was obtained.

Although later Zhang et al.[6] successfully synthesized 3,N^4-ethano-dC (**3**) (Chart 1), the procedure was not suitable as a synthetic route to prepare the 1,N^6-ethano-dA (**1**). In using this procedure, it was observed that when the silylated deoxyinosine **7** was treated with 4-chlorophenyl phosphorodichlorate in the presence of 1,2,4-triazole in pyridine (Scheme 1), a fluorescent material was obtained as the main product, whereas the desired 6-(3-nitro-1,2,4-triazol-1-yl) derivative **8** was recovered only as a trace component.[10] An earlier report[11] suggested the formation of fluorescent *N*-(purin-6-yl)pyridinium salts, such as **9**, by phosphorylations in pyridine. It was also found that the substitution of the 3-nitro-1,2,4-triazol moiety of **8** by 2-chloroethylamine did not proceed as expected, and upon heating only traces of the cyclized product **10** in a complex mixture were detected.[10] Since our results showed that the spontaneous ring closure is not as easily attainable as in the case of ethano-dC[6], we did not continue this route.

However, using a new and different synthetic route, the desired compound **1** was obtained in high yield and purity. The 6-chloropurine-2′-deoxyriboside **11** was prepared according to the procedure published by Robins and Basom.[12] This compound **11** was then derivatized to give compound **12** in 86% yield using *tert*-butyldimethylsilyl chloride (Scheme 2), which was reacted with 2-hydroxyethylamine to produce compound **13** in 64% yield. Iodination of the β-hydroxyl group of **13** with methyltriphenoxyphosphonium iodide[13] in DMF at room temperature for 30 min led to the spontaneous cyclization of the

(7) Zhang, W.; Johnson, F.; Grollman, A. P.; Shibutani, S. *Chem. Res. Toxicol.* **1995**, *8*, 157−163.
(8) Ludlum, D. B. *Pharmac. Ther.* **1987**, *34*, 145−153.
(9) Tong, W. P.; Ludlum, D. B. *Biochem. Pharmacol.* **1979**, *28*, 1175−1179.
(10) Low-resolution mass spectroscopy on purified material was consistent with structure.
(11) Adamaiak, R. W.; Biala, E. New. *Nucleic Acids Res.* **1985**, *13* (8), 2989−3003.
(12) Robins, M. J. and Basom, G. L. *Can. J. Chem.* **1973**, *51*, 3161−3169.

Scheme 2. Synthesis of the Phosphoramidite of 1,N^6-Ethano-dA Adduct



resulting N^6-(2-iodoethyl) derivative, producing the 1,N^6-ethano-derivative **10** in 67% yield. Desilylation of the latter was accomplished by a treatment with triethylamine trihydrofluoride[14] in THF. This protocol allowed almost quantitative recovery of 1,N^6-ethano-dA (**1**) in 91% yield, whereas the typical tetrabutylammonium fluoride silyl deprotection procedure[6] necessitated several purification steps to free the product from tetrabutylammonium fluoride residue. The 1,N^6-ethano-dA (**1**) synthesized by this method was analyzed by TLC, HPLC, ^{1}H NMR, ^{13}C NMR, UV, electrospray/MS, FAB/MS, and high-resolution MS (see the Experimental Section). The UV of this compound **1** showed the same UV spectral characteristics as that described by Tong and Ludlum:[3,9] pH 7 (λ_{max} 262, λ_{min} 235), pH 1 (λ_{max} 262, λ_{min} 235), and pH 13 (λ_{max} 269, λ_{min} 239). The FAB/MS of product **1** showed a protonated molecular ion at *m*/*z* 580 MH^+ and protonated base at *m*/*z* 162 BH_2^+. All the data collected were in agreement with the structure of 1,N^6-ethano-dA (**1**). This compound proved to be stable to all the reagents and conditions used in the automated DNA synthesis, and therefore its phosphoramidite was prepared.

Synthesis of the Phosphoramidite of 1,N^6-Ethano-2′-deoxyadenosine (15) and Its Site-Specific Incorporation into a DNA Oligonucleotide. Compound **1** has two hydroxy groups (3′- and 5′-OH) on the sugar moiety. To incorporate this adduct in an oligonucleotide, all reactive hydroxy groups must be protected. The first attempt to tritylate compound **1** according to the standard methods with DMTCl[16,17] failed. However, the treatment of compound **1** with $DMT^+BF_4^-$ [18] yielded the 5′-O-DMT product **14** in 62% yield in 1 h. Phosphitylation of 5′-*O*-DMT-1,N^6-ethano-dA (**14**) with 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite[19] yielded a 1:1 mixture of the expected diastereoisomers **15** in 71% yield. This product **15** was coevaporated three times from dry benzene and kept in a vacuum desiccator over P_2O_5 and NaOH pellets before being used to make the desired oligonucleotide. The fully protected compound **15** was used to synthesize a 25-mer oligonucleotide as shown in the Experimental Section. The commercially available normal phenoxyacetyl phosphoramidite nucleotides (PAC) were used in the synthesis of this DNA oligomer and followed standard phosphoramidite chemistry. The overall yield was 87%, and the coupling efficiency of 1,N^6-ethano-dA phosphoramidite was 93%. The oligonucleotide was then deprotected under aqueous conditions by using 28−30% ammonia for 1.5 h at 65 °C and then purified by HPLC. The use of the PAC protecting group allowed the deprotection of DNA oligomer in a shorter time (1.5 h) than the standard phosphoramidite, which requires several hours (8−16 h).

The composition of the DNA oligomer was confirmed after enzymatic digestion to nucleosides, followed by HPLC analysis, which showed that the modified base survived the conditions used in the DNA synthesizer and the deprotection procedure (Figure 1).

This new oligonucleotide-containing 1,N^6-ethano-2′-deoxyadenosine is being used to obtain information about the repair, mutagenesis, and structural properties of this

(13) Saito, T.; Murakami, M.; Inada, T.; Hayashibara, H.; Fujii, T. Purines. LIV. *Chem. Pharm. Bull.* **1993**, *41* (3), 453−457.
(14) Pirrung, M. C.; Shuey, S. W.; Lever, D. C.; Fallon, L. A. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1345−1346.
(15) Ogilvie, K. K.; Beaucage, S. L.; Schifman, A. L.; Theriault, N. Y.; Sadana, K. L. *Can. J. Chem.* **1978**, *56*, 2770−2780.
(16) Schaller, H.; Weimann, G.; Lerch, B.; Khorana, H. G. *J. Am. Chem. Soc.* **1963**, *85*, 3821−3827.
(17) Chaudhary, S. K.; Hernandez, O. *Tetrahedron Lett.* **1979**, *2*, 99−102.
(18) Bleasdale, C.; Ellswood, S. B.; Golding, B. T. *J. Chem. Soc., Perkins. Trans. 1* **1990**, 803−805.
(19) Bodepudi, V.; Shibutani, S.; Johnson, F. *Chem. Res. Toxicol.* **1992**, *5*, 608−617.

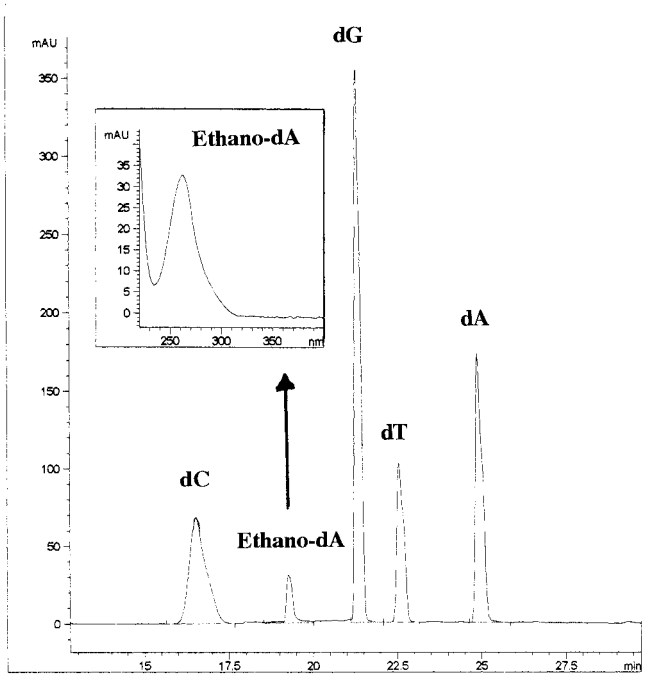


Figure 1. HPLC profile of 2′-deoxynucleosides obtained as a result of enzymatic digestion of the oligonucleotide that contains 1,N^6-ethano-dA (Experimental Section). HPLC was performed using system 2. The retention times of the deoxynucleosides are dC, 16.5 min; 1,N^6-ethano-dA, 19.3; dG, 21.3 min; dT, 22.6 min; and dA, 24.9 min. The UV spectrum of 1,N^6-ethano-dA at pH 4.5 is shown in the inset.

adduct. This should lead to a better understanding of the cytotoxic and carcinogenic mechanisms of the effect of BCNU.

In summary, 1,N^6-ethano-dA, which is one of many adducts formed from the reaction of the chemotherapeutic agent 1,3-bis(2-chloroethyl)nitrosourea (BCNU) and DNA, was synthesized on a large scale for the first time and converted to its phosphoramidite. This compound was then incorporated into a site-specific deoxyoligonucleotide to be used for biochemical and physical studies.

Experimental Section

General Procedures. ^{1}H, ^{13}C, and ^{32}PNMR spectra were recorded in $CDCl_3$, unless stated otherwise. Fast atom bombardment (FAB) mass spectra were run in glycerin or thioglycerin as matrix. Ultraviolet spectra were recorded in a spectrophotometer using 0.5 cm cuvettes. TLC was performed on EM 5735/7 silica gel 60, F_{254} plates. Flash column chromatography was carried out using silica gel 60, eluting with the solvents indicated. Compounds characterized in this manner were homogeneous by TLC analysis and gave NMR spectra indicative of their purity. 6-Chloro-9-(2′-deoxypentafuranosyl)-purine[12] and 4,4′-dimethoxytrityl tetrafluoroborate[18] were prepared following published procedures. 2′-Deoxyinosine was purchased from Sigma. Methylene chloride, benzene, pyridine, and triethylamine were dried by distilling over CaH_2. All other anhydrous reagents and remaining chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI) and used without further purification.

HPLC. Solvent systems included solvent A (acetonitrile), solvent B (triethylammonium acetate; 0.1 M, pH 7.0), and solvent C (potassium phosphate buffer; 0.01 M, pH 4.5). **System 1.** The oligonucleotide was purified using a PRP-1 C-18 semipreparative reversed-phase column (Hamilton Co.). The initial concentration was 10% solvent A, 90% solvent B, with solvent A linearly increased to 40% over 30 min at a flow rate of 2 mL/min. **System 2.** Analysis of the enzyme digest of the oligonucleotide was performed using a Supelcosil LC-18-DB column (2.5 cm × 0.46 cm, 5 μm, Supelco, Inc.) with 0% solvent A and 100% solvent C. Solvent A was maintained isocratic for 10 min and then was increased linearly to 12% over 30 min, at a flow rate of 1 mL/min.

6-Chloro-9-[3′,5′-*O*-bis(*tert*-butyldimethylsilyl)-2′-deoxypentafuranosyl]purine (12). 6-Chloro-9-(2′-deoxypentafuranosyl)purine[12] (**11**) (0.97 g, 3.6 mmol), *tert*-butyldimethylsilyl chloride (2.39 g, 4.4 equiv), and imidazole (2.16 g, 8.8 equiv) were dissolved in dry DMF (3 mL), and the mixture was stirred at room temperature for 3 h. The solution was then poured into 60 mL of water and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extracts were washed with brine (30 mL) and dried over $MgSO_4$, and the residue, obtained after evaporation of the organic solvent, was purified by flash column chromatography (CH_2Cl_2/MeOH/Et_3N 20:0.1:0.1). The product was obtained as a light oil in 86% yield (1.53 g): ^{1}H NMR δ 0.05 (s, 6H, $Si(CH_3)_2$), 0.07 (s, 6H, $(CH_3)_2$), 0.86 (s, 9H, $SiC(CH_3)_3$), 0.90 (s, 9H, $SiC(CH_3)_3$), 2.45 (m, 1H, H-2′), 2.60 (m, 1H, H-2′), 3.81 (dd, 2H, H-5′), 4.02 (m, 1H, H-4′), 4.65 (m, 1H, H-3′), 6.45 (t, 1H, H-1′), 8.56 (s, 1H, H-8), 8.78 (s, 1H, H-2); ^{13}C NMR δ −5.32, −5.52, −4.89, −4.71, 17.95, 18.39, 25.69, 25.91, 41.57, 43.53, 71.76, 84.87, 88.17, 132.31, 143.77, 150.81, 151.22, 151.81; HRMS calcd for $C_{22}H_{40}N_4O_3Si_2Cl$ [M + H$^+$] 499.232 751, found 499.233 010; LRMS (FAB$^+$) *m/e* 499 [M + H]$^+$, 155 [base + 2H]$^+$; R_f 0.35 (CH_2Cl_2/MeOH/Et_3N 20:0.1:0.1).

6-*N*-Hydroxylamino-9-[3′,5′-*O*-bis(*tert*-butyldimethylsilyl)-2′-deoxypentafuranosyl]purine (13). Compound **12** (0.77 g, 1.5 mmol) was dissolved in anhydrous methanol (7 mL). Ethanolamine (0.47 g, 5 equiv, 0.46 mL) was added dropwise and the solution allowed to reflux for 2 h. The solvent was evaporated under reduced pressure, and the resulting oil was dissolved in CH_2Cl_2 (25 mL) and washed with saturated brine (3 × 10 mL). The organic extract was dried over $MgSO_4$, and after filtration and concentration the residue was purified by flash column chromatography (CH_2Cl_2/MeOH/Et_3N 19.25:0.75:0.1) to give 0.5 g (64%) of **13** as an off-white oil: ^{1}H NMR δ 0.05 (s, 6H, $Si(CH_3)_2$), 0.07 (s, 6H, $Si(CH_3)_2$), 0.86 (s, 9H, $SiC(CH_3)_3$), 0.90 (s, 9H, $SiC(CH_3)_3$), 2.43 (m, 1H, H-2′), 2.58 (m, 1H, H-2′), 3.77 (m, 2H, H-5′), 3.86 (m, 2H, CH_2-NH), 3.89 (m, 2H, CH_2OH), 3.99 (m, 1H, H-4′), 4.59 (m, 1H, H-3′), 5.85 (br, 1H, CH_2OH), 6.42 (t, 1H, H-1′), 6.94 (br, 1H, CH_2NH), 8.16 (s, 1H, H-8), 8.35 (s, 1H, H-2); ^{13}C NMR δ −5.60, −5.52, −4.94, −4.76, 17.87, 18.29, 25.63, 25.84, 41.32, 43.60, 61.89, 62.48, 71.42, 84.23, 87.67, 110.39, 119.63, 138.04, 152.76, 154.80; HRMS calcd for $C_{24}H_{46}N_5O_4Si_2$ [M + H]$^+$ 524.308 837, found 524.308 490 LRMS (FAB$^+$) *m/e* 524 [M + H]$^+$, 180 [base + 2H]$^+$; R_f 0.26 (CH_2Cl_2/MeOH/Et_3N 19.25:0.75:0.1).

1,N^6-Ethano-9-[3′,5′-*O*-bis(*tert*-butyldimethylsilyl)-2′-deoxypentafuranosyl]purine (10). Compound **13** (0.8 g, 1.5 mmol) was dissolved in anhydrous DMF (6 mL) containing Et_3N (5 equiv, 1.0 mL). The solution, cooled in ice bath, was treated with methyltriphenoxyphosphonium iodide (1.6 g, 2.5 equiv) and stirred at room temperature for 45 min. The mixture was then treated with anhydrous MeOH (2 mL) to quench the residual phosphonium reagent. All solvents were evaporated, and the oily residue was dissolved in CH_2Cl_2 (10 mL) and washed with $NaHCO_3$ (5%, 5 mL) and saturated brine (3 × 5 mL). The organic phase was then dried over $MgSO_4$, concentrated to dryness, and purified by column chromatography (CH_2Cl_2/MeOH/Et_3N 18:1:0.1) with a yield of 67% (0.52 g): ^{1}H NMR (DMSO-d_6) δ 0.06 (s, 6H, $Si(CH_3)_2$), 0.07 (s, 6H, $Si(CH_3)_2$), 0.81 (s, 9H, $SiC(CH_3)_3$), 0.85 (s, 9H, $SiC(CH_3)_3$), 2.38 (m, 1H, H-2′), 2.78 (m, 1H, H-2′), 3.63 (dd, 1H, H-5′), 3.77 (dd, 1H, H-5″), 3.87 (m, 1H, H-3′), 4.07 (t, 2H, J = 9.5 Hz, CH_2N-), 4.58 (m, 1H, H-4′), 4.68 (t, 2H, J = 9.5 Hz, CH_2N=), 6.35 (t, 1H, J = 6.5 Hz, H-1′), 8.65 (s, 1H, H-8), 8.76 (s, 1H, H-2); ^{13}C NMR δ −5.44, −5.33, −4.84, −4.63, 17.92, 18.38, 25.69, 25.94, 41.78, 44.17, 47.08, 62.69, 71.63, 85.30, 88.44, 117.01, 142.50, 143.52, 148.13, 151.11; HRMS calcd for $C_{24}H_{44}N_5O_3Si_2$ [M +

H]$^+$ 506.298 273, found 506.298 020; LRMS (FAB$^+$) *m/e* 506 [M + H]$^+$, 162 [base + 2H]$^+$; R_f 0.30 (CH_2Cl_2/MeOH/Et_3N 16:1:0.1).

1,N^6-Ethano-9-(2′-deoxypentafuranosyl)purine (1). Compound **10** (0.26 g, 0.52 mmol), dissolved in anhydrous THF (2 mL), was treated with triethylamine trihydrofluoride (332 μL, 2.01 mmol) and stirred at room temperature for 1 h. The white slurry formed was evaporated to dryness, dissolved in water, and neutralized with $NaHCO_3$. The water was evaporated, and the white solid was purified by flash column chromatography (CH_2Cl_2/MeOH/Et_3N 15:5:0.2) with a yield of 91% (0.13 g): ^{1}H NMR (DMSO-d_6) δ 2.31 (m, 1H, H-2′), 2.58 (m, 1H, H-2′), 3.56 (m, 2H, H-5′), 3.86 (q, 1H, H-4′), 4.41 (m, 1H, H-3′), 3.99 (t, 2H, J = 10 Hz, CH_2N−), 4.45 (t, 2H, J = 9.6 Hz, CH_2N═), 5.02 (br, 1H, 5′- OH), 5.37 (br, 1H, 3′-OH), 6.31 (t, 1H, H-1′), 8.49 (s, 1H, H-8), 8.50 (s, 1H, H-2); ^{13}C NMR (D_2O; TMS, ext standard) δ 39.03, 44.66, 48.22, 61.27, 70.78, 84.91, 87.51, 116.57, 143.42, 144.48, 148.96, 151.37; HRMS calcd for $C_{12}H_{16}N_5O_3$ [M + H]$^+$ 278.125 315, found 278.125 920; LRMS (ES$^+$) *m/e* 278 [M + H]$^+$, 162 [base + 2H]$^+$; R_f 0.28 (CH_2Cl_2/MeOH/Et_3N 15:5:0.2).

1,N^6-Ethano-9-[5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxypentafuranosyl]purine (14). Nucleoside **1** (25 mg, 0.09 mmol), DMT$^+BF_4^-$ (35 mg, 1 equiv), and Li_2CO_3 (14 mg, 2 equiv) were combined and dried under vacuum for 30 min. 2,6-Lutidine (1 mL) was added, and the solution was allowed to stir at room temperature for 3 h. The reaction was monitored by TLC, which indicated the presence of starting material. More DMT$^+BF_4^-$ (35 mg, 1 equiv) was then added, and the reaction mixture was stirred at room temperature for another 3 h period. The mixture was diluted with CH_2Cl_2 (10 mL) and filtered over MeOH (1 mL). The solvents were evaporated, and the residue was purified by flash column chromatography (CH_2Cl_2/MeOH/Et_3N 18.5:1.5:0.1) to yield 32 mg (62%) of a colorless oil: ^{1}H NMR δ 2.50 (m, 1H, H-2′), 2.83 (m, 1H, H-2′), 3.28 (m, 2H, H-5′), 3.69 (s, 6H, OCH_3), 3.97 (m, 1H, H-4′), 4.10 (m, 1H, H-3′), 4.14 (t, 2H, J = 4.6 Hz, CH_2N−), 4.60 (t, 2H, J = 5.4 Hz CH_2N═), 5.37 (br, 3′-OH), 6.28 (t, J = 6.3 Hz, 1H, H-1′), 6.71 (dd, J = 8.9, 2.0 Hz, 4H, H ortho to OCH_3), 7.09−7.35 (m, 9H, ArH), 7.66 (s, 1H, H-8), 7.76 (s, 1H, H-2); ^{13}C NMR δ 40.59, 46.56, 52.28, 55.06, 63.83, 71.19, 84.24, 86.09, 86.23, 112.89, 119.64, 126.59, 127.60, 127.99, 129.92, 129.96, 135.65, 137.56, 143.17, 144.57, 151.46, 158.27; HRMS calcd for $C_{33}H_{34}N_5O_5$ [M + H]$^+$ 580.255 995, found 580.255 410; LRMS (FAB$^+$) *m/e* 580 [M + H]$^+$, 162 [base + 2H]$^+$, R_f 0.27 (CH_2Cl_2/MeOH/Et_3N 18.5:1.5:0.1).

1,N^6-Ethano-9-[5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-[(*N*,*N*-diisopropylamino)(β-cyanoethoxy)phosphinyl]-2′-deoxypentafuranosyl]purine (15). The 5′-DMT-protected nucleoside **14** (68 mg, 0.12 mmol), dried over P_2O_5 and KOH over high vacuum for 12 h, was dissolved in anhydrous CH_2Cl_2 (2 mL) and anhydrous Et_3N (218 μL, 12 equiv) under N_2 atmosphere. 2-Cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (87 μL, 3 equiv) was added dropwise and the reaction mixture stirred at room temperature for 1.5 h. It was then treated with anhydrous MeOH (200 μL), diluted with CH_2Cl_2 (5 mL), and washed with brine (2 × 3 mL). The organic layer was dried over $MgSO_4$, evaporated to dryness, and purified by flash column chromatography (CH_2Cl_2/MeOH/Et_3N 19:0.5:0.1) to give the product as a light yellow oil (65 mg) in 71% yield: ^{31}P NMR δ 149.37, 149.43 (diastereomeric pair); HRMS calcd for $C_{42}H_{51}N_7O_6P$ [M + H$^+$] 780.363 846, found 780.365 110; LRMS (FAB+) *m/e* 780 [M + H]$^+$, 162 [base + 2H]$^+$; R_f 0.24 (CH_2Cl_2/MeOH/Et_3N 19:0.5:0.1).

Solid-Phase Synthesis of the Oligonucleotide. The phosphoramidite of compound **15** (65 mg) was coevaporated three times from dry benzene then was dried over P_2O_5 and NaOH pellets in a vacuum desiccator for 48 h at room temperature. This material was dissolved under dry nitrogen in 2 mL of dry acetonitrile and used to synthesize the DNA oligomer. Synthesis of the oligonucleotide was performed on an Applied Biosystem 392 automated DNA synthesizer on a 1 μmol scale using phosphoramidite chemistry on a controlled pore glass (CPG) support with an aminopropyl succinate linker. Phenoxyacetyl phosphoramidites (PAC) from Pharmacia were used to synthesize the DNA oligomer. The synthesis followed standard protocol of the DNA synthesizer for phosphoramidite chemistry, except for the two steps in which the modified deoxynucleoside and the base after were inserted. In the steps where the modified nucleotide and the following unmodified nucleotide were incorporated, the time of coupling was increased to 16 min and 10 min, respectively, to maximize the coupling efficiency, which was 93%. The overall yield of the synthesis of the oligonucleotide was 87%, which was recovered as the 5′-dimethyloxytritylated derivative and deprotected with 28−30% aqueous ammonia for 1.5 h at 65 °C to cleave off the nucleobase protecting groups. The 5′-DMT-oligomer was purified by HPLC and eluted at 20 min using a PRP-1 C-18 semipreparative reversed-phase column (Hamilton Co.) using system 1. Detritylation of the pure oligomer was accomplished by the treatment with 80% acetic acid for 20 min at room temperature and then was desalted on a Sep-pack C-18 column. Finally, the product was lyophilized on a Speed-Vac evaporator and dissolved in 1 mL of deionized water. The amount of DNA measured using UV spectrometry was 19.6 A_{260} units/1 mL. The resulting fully deprotected DNA containing the modified base was reanalyzed by HPLC and found to be a single product that has the following sequence:

5′-CCGCT**X**GCGGGTACCGAGCTCGAAT-3′

X = 1,N^6-Ethano-A

This sequence was originally taken from the polylinker region of M13 phage. It has been used extensively in this laboratory for mutagenesis and repair studies.

To confirm the compostion of the oligomer, about 0.45 A_{260} units was dissolved in 44 μL of freshly deionized water, 0.8 μL of 1 M $MgCl_2$, and 3.5 μL 0.5 M Tris−HCl buffer (pH 7.5) and digested with snake venom phosphodiesterase (2.5 μL) and bacterial alkaline phosphatase (4.0 μL) at 37 °C for 16 h. The digested mixture was analyzed on a C-18 reversed-phase HPLC Supelcosil column (250 × 4.5 mm) using system 2. Quantitation of the deoxynucleosides was on the basis of integration of the peak areas at 260 nm, which confirmed the base composition and the incorporation of the adducts, as well as the spectrum of 1,N^6-ethano-dA (Figure 1).

Acknowledgment. This work was supported by Grant No. CA47723 from the National Institutes of Health to B.S. and was administered by the Lawrence Berkeley National Laboratory under Department of Energy Contract No. DE-AC03-76SF00098.

JO980170I