Synthesis of Enzymatically Noncleavable Carbocyclic Nucleosides for DNA−*N*-Glycosylase Studies

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Received August 25, 1997

Carbocyclic nucleosides have been of great interest as antiviral agents and in studies in the area of antisense technology. The recent finding that the replacement of a single 2′-deoxynucleoside in DNA by a carba analogue does not alter the Watson−Crick base pairing, yet at the same time provides a chemically and enzymatically stable "glycosidic" linkage, led us to examine this class of compound as enzyme inhibitors of the DNA-repair enzymes involved in oxidative damage. We now report the synthesis and incorporation into oligomeric DNA via suitable derivatives, the carbanucleosides 8-oxo-7,8-dihydro-2′-deoxycarbainosine, 8-oxo-7,8-dihydro-2′-deoxycarbaguanosine, and 2′-deoxyaristeromycin. Aristeromycin (**1**) was deoxygenated at the 2′-position as follows. Treatment of **1** with $TPDSCl_2$ gave the 3′,5′-protected derivative **3** (76%) which on phenylthiocarbonylation at the 2′-position gave **4** in 51% yield. The latter compound on reduction with Bu_3SnH led to the 2′-deoxy derivative **5** (90%). Benzoylation followed by deprotection with TBAF in THF then gave the desired intermediate (**6**) in 65% yield. N^2-Isobutyryl-8-oxo-7,8-dihydro-2′-deoxycarbaguanosine (**16**) was synthesized from 3-chloro-2′-deoxycarbainosine (**9**). Treatment of **9**, either with hydrazine followed by catalytic reduction of the 2-hydrazino derivative or with 1-(2-nitrophenyl)ethylamine followed by photolysis of the resulting 2-substituted derivative, in both instances gave the desired 2′-deoxycarbaguanosine (**12**) in ∼50% overall yield in each case. Bromination of **12** gave **13** (90%) which, when treated with BnONa in DMSO at 65 °C, led to the 8-benzyloxy derivative **14** (46%). Isobutyrylation of **14** followed by catalytic reduction then afforded **16**. 8-Oxo-7,8-dihydro-2′-deoxycarbainosine (**23**) was prepared in four steps. Bromination of 2′-deoxyaristeromycin (**19**) at the 8-position gave **20** (>95%) which was converted to the 8-benzyloxy derivative **21** (61%) using BnONa/DMSO at 80 °C. Reductive debenzylation of **21** then led to 8-oxo-7,8-dihydro-2′-deoxyaristeromycin (∼100%) which, when treated with adenosine deaminase, provided the desired carbainosine derivative **23** in quantitative yield. Compounds **6**, **16**, and **23** were converted to their respective 5′-*O*-DMT, 3′-*O*-[(2-cyanoethoxy)-(*N*,*N*-diisopropylamino)-phosphine] derivatives (**8**, **18,** and **25**) in excellent overall yields. The latter were then used to synthesize a series of DNA oligomers by automated procedures.

Introduction

DNA damage, induced by free radicals, ionizing radiation, or environmental toxicants, causes mutation in DNA and if unrepaired leads frequently to alteration of gene function which may result in cancer (*1*). In the DNA-repair processes there are several glycosylase enzymes which recognize and cleave primarily the damaged bases from DNA (*2*). In the study of these processes, significant synthetic efforts have been made to incorporate the modified nucleosides representing the damage into oligomeric DNA in a site-specific manner. The objectives in any one case are to examine the damage recognition process, to uncover the mechanistic details of the base cleavage, and to detail the precise manner in which the enzyme manipulates the gross DNA structure while effecting the repair. These aims pose significant problems because the action of the bound enzyme is difficult to impede, except by lowering the temperature, by the use of a tight-binding product mimic, or more rarely by the omission of some vital cofactor such as magnesium. Compounding the problem is the fact that certain DNA lesions are not hydrolytically stable at the glycosidic linkage, so their incorporation into oligomeric DNA by standard solid-phase techniques is not feasible. The hydrolytic susceptibility of this linkage can be diminished by introducing an electronegative group at the 2′-position as is readily evident from the greater stability of ribonucleosides compared with their 2′-deoxy analogues. The 2′-fluoro-substituted derivatives also share enhanced stability (*3*).

Since the completion of the current work, others have shown that the problem of hydrolytic (and enzymatic) instability of the deoxynucleosides may be solved either by inserting a methylene group between the heterocyclic moiety and the furanose ring (*4*) or by the use of modified nucleosides such as 2′-deoxytubercidin or 2′-deoxyformycin (*5*). However, so far these approaches have been limited to mimics of 2′-deoxyadenosine.

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S0893-228x(97)00153-7 CCC: $15.00
Published on Web 02/07/1998

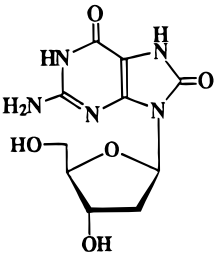
It appeared to us, nevertheless, that this endemic problem of the hydrolytic instability of the glycosidic linkage might be solved simply and generally by replacing the furanose ring oxygen atom by carbon. This removes the element responsible for the instability of the O−C−N bond system (hemiamine acetal) and generates a C−C−N group that is very stable to chemical modification yet, at the same time, should have little influence on the ability of the repair enzyme to bind to DNA. Several compounds of this class, the carbocyclic nucleosides, were originally developed as antiviral agents (*6*, *7*) either by direct total synthesis or by transformation from aristeromycin (**1**, Aris)[1] which is available by fermentation (*8*). During the past 2 years, new totally synthetic routes (*9*−*11*) have been developed making these unnatural nucleosides readily available thus enhancing the opportunity to incorporate them into DNA.

The rapidly growing application of carbocyclic nucleosides in DNA research is in the area of antisense technology (*12*). This new potentially therapeutic approach aims to control protein synthesis, which specifically is involved in diseases, at the DNA or RNA level by using sugar or base modifications to increase stability toward nucleases and against simple depurination. Moser has reported extended stability studies (*13*) on a complete carbocyclic sequence annealed to an RNA strand and separately to a DNA strand. The chemical stability of the carbaDNA was found to be enhanced. He also studied the melting behavior of carbaDNA−DNA and carbaDNA−RNA complexes to determine the stability of the unnatural double strands. The T_M value was slightly decreased (−0.4 °C/base) in the carbaDNA−DNA case, whereas the stability of carbaDNA−RNA was slightly higher than the unmodified form (DNA−RNA). From these results, it appears that a single carbanucleoside replacement in DNA does not alter the Watson−Crick base pairing (*14*) yet provides a chemically and enzymatically stable entity suitable to study those processes which normally lead to the cleavage of the glycosidic linkage. This strategy was used first to identify intermediates involved in the proposed mechanism (*15*) of DNA cleavage by bleomycin. In this case, a single carbocyclic adenosine (2′-deoxyaristeromycin, dAris, **1**) residue was site-specifically introduced into DNA (*16*).

Our research on DNA repair currently is focused primarily on oxidative damage, specifically the C-8 oxidation product 8-oxo-7,8-dihydro-2′-deoxyguanosine (**2**; Chart 1) (*17*). This lesion is recognized principally by MutM enzyme (FaPy-DNA glycosylase), which cleaves it efficiently. On the other hand, during DNA replication, unrepaired 8-oxo-dG residues, besides being paired with a dC residue, also form a mismatched base pair with 2′-deoxyadenosine. This latter lesion is repaired by MutY enzyme, which excises the opposite base (dA) creating a gap, which in the subsequent iterative repair process is filled with either a 2′-dC (correct) or repetitively with a dA (incorrect) residue (Scheme 1).

[1] Abbreviations. Compounds: Aris, aristeromycin; dAris, 2′-deoxyaristeromycin; 8-oxo-dG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; dA, 2′-deoxyadenosine; dC, 2′-deoxycytidine; carba-dG, 2′-deoxycarbaguanosine; 8-oxo-dI, 8-oxo-2′-deoxyinosine; 8-oxo-carba-dI, 8-oxo-2′-deoxycarbainosine. Reagents: AIBN, 2,2′-azobis(isobutyronitrile); DMT-Cl, 4,4′-dimethoxytrityl chloride; DMSO, dimethyl sulfoxide; TBAF/THF, tetrabutylammonium fluoride in tetrahydrofuran; TPDS-Cl_2, 1,3-dichlorotetraisopropyldisiloxane.

Chart 1. Major Oxidative Lesion Occurring in Native DNA



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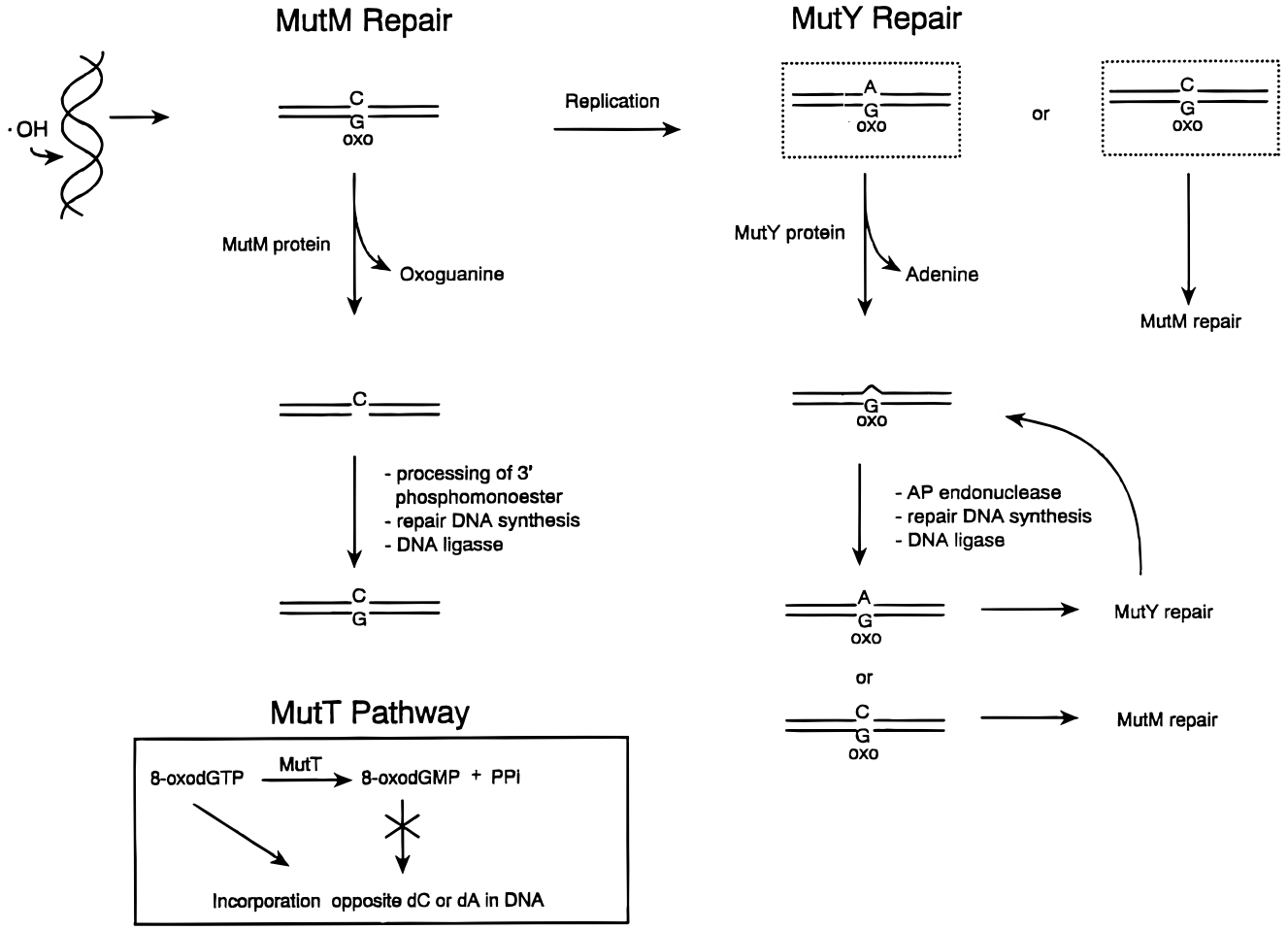
Experimental Procedures

Dry pyridine and acetonitrile were distilled from calcium hydride. DMT-Cl was recrystallized from 5 volumes of cyclohexane/acetyl chloride (10:1). ^{1}H and ^{13}C NMR spectra were obtained from a Bruker AC-250 or General Electric QE 300 spectrometer. FAB mass spectra were recorded on a Kratos MS-890/DS-90 instrument using 50% glycerol/thioglycerol as the matrix. Electrospray mass spectra were taken on a Micromass Trio 2000 spectrometer. High-resolution mass spectra were obtained from the UCR Mass Service Facility, Department of Chemistry, University of California, Riverside, CA. The purity of all new compounds was judged to be better than 97% by TLC analysis in two solvent systems, either chloroform/methanol or ethyl acetate/ethanol.

Synthesis of 2′-Deoxyaristeromycin. 3′,5′-*O*-TPDS-aristeromycin (3). An amount of 540 mg (2.1 mmol) of aristeromycin (**1**) was partially dissolved in freshly distilled, dry pyridine (20 mL) under nitrogen by means of sonication. The resulting white suspension was then treated with TPDS-Cl_2 (0.74 mL, 2.3 mmol), and the mixture was stirred for 12 h, when it became clear. The pyridine was evaporated to dryness giving a yellow foamy syrup, and water (10 mL) was added followed by ethyl acetate (100 mL); with vigorous stirring, the two-phase system was made acidic with 0.2 M $NaHSO_4$, and the phases were separated. The organic layer was immediately washed with water (15 mL), saturated $NaHCO_3$ solution (15 mL), then water (15 mL), and finally brine. The organic extract was dried over $MgSO_4$ and evaporated in vacuo giving a glassy foam (1.33 g). The crude material was purified on a silica gel column (110 g), elution being accomplished with chloroform/methanol (100:5). The required product **3** (810 mg) was obtained as a viscous colorless oil in 76% yield. ^{1}H NMR (250 MHz, δ, $CDCl_3$): 8.25 (s, 1 H, H-8), 7.82 (s, 1 H, H-2), 5.78 (brs, 2 H, NH_2); 4.64−4.72 (m, 1H, H-1′); 4.61−4.64 (dd, 1H, H-2′); 4.35−4.39 (m, 1H, H-3′), 4.02 and 3.99 (2 × dd, 2 H, H-5′), 3.15 (brs, 1 H, OH), 2.25 (m, 1H, H-4′), 1.90−2.11 (m, 2 H, *carba*-CH_2), 0.95−1.1 (m, 28 H, isopropyl). ^{13}C NMR (63 MHz, δ, $CDCl_3$): 157.0, 152.6, 139.9, 75.5, 72.0, 61.8, 60.9, 45.8, 29.1, 17.5, 17.3, 17.1, 13.4, 12.9. HRMS (DCI, NH_3): calcd for $C_{23}H_{42}O_4Si_2N_5$ $(M + H)^+$ at *m*/*z* 508.2775, found 508.2770.

2′-*O*-[(Phenoxythio)carbonyl]-3′,5′-*O*-TPDS-aristeromycin (4). 3′,5′-*O*-TPDS−aristeromycin (**3**; 375 mg, 0.74 mmol) was dissolved in dry acetonitrile (8 mL) under nitrogen at 55 °C. To this solution were added *N*,*N*-(dimethylamino)pyridine (253 mg, 2.07 mmol) and phenyl chlorothionoformate (0.105 mL, 0.75 mmol). The yellow solution was stirred at 55 °C. After 10 h, the acetonitrile was removed in vacuo, and the resulting yellow powder was dissolved in methylene chloride and washed successively with 0.2 M $NaHSO_4$ (10 mL), saturated $NaHCO_3$ (2 × 10 mL), water (10 mL), and then brine. The organic extract was dried over $MgSO_4$ and evaporated in vacuo giving a yellow oil. The crude material (610 mg) was purified by silica gel column chromatography [silica gel, 45 g; mobile phase, chloroform/methanol (100:2.5)] to give title compound **4** in 90% yield (432 mg). ^{1}H NMR (250 MHz, δ, $CDCl_3$): 8.23 (s, 1 H, H-8), 7.78 (s, 1 H, H-2), 7.35−7.42 (m, 2 H, phenyl), 7.26 (t, 1 H, phenyl), 7.08 (d, 2 H, phenyl), 6.06 (m, 1 H, H-2′), 5.96 (brs, 2 H, NH_2), 5.09 (m, 1 H, H-1′), 4.91 (1 H, m, H-3′), 4.05 and 3.85 (2 × dd,

Scheme 1[a]



[a] Grollman, A. P., and Moriya, M. (1993) *Trends Genet.* **9**, 246−249. Reproduced by kind permission of the Editor.

2 H, H-5′), 2.21−2.27 (m, 3 H, H-4′, *carba*-CH_2), 0.93−1.15 (m, 28 H, isopropyl). ^{13}C NMR (63 MHz, δ, $CDCl_3$): 157.1, 153.0, 152.5, 150.2, 140.2, 129.5, 126.6, 121.8, 120.3, 86.3, 70.8, 61.6, 61.4, 45.6, 29.1, 17.5, 17.3, 17.1, 13.4, 12.9. HRMS (FAB, NBA): calcd for $C_{30}H_{46}O_5Si_2N_5S$ $(M + H)^+$ at *m*/*z* 644.2758, found 644.2751.

2′-Deoxy-3′,5′-*O*-TPDS-aristeromycin (5). A solution of dry 2-*O*-[(phenoxythio)carbonyl]-3′,5′-*O*-TPDS-aristeromycin (**4**; 240 mg, 0.237 mmol) in freshly distilled toluene (7.5 mL) was degassed by repeated gentle evacuation followed by bubbling nitrogen through the solution for 20 min. To the solution were then added tributyltin hydride (0.15 mL, 0.56 mmol) and dry AIBN (12 mg). The reaction mixture was stirred at 75 °C for 3 h under nitrogen. Thereafter, the colorless liquid was evaporated to dryness, and the residue was dissolved in chloroform and applied directly onto a silica gel-packed column [mobile phase, chloroform/methanol (100:2.5)]. Evaporation of the combined fractions gave the desired compound **5** (0.17 g, yield 91%) as a crystalline solid, mp 110−111 °C. ^{1}H NMR (250 MHz, δ, $CDCl_3$): 8.30 (s, 1 H, H-8), 7.80 (s, 1 H, H-2), 5.68 (brs, 2 H, NH_2), 5.05 (m, 1 H, H-1′), 4.67 (m, 1 H, H-3′), 4.03 and 3.77 (2 × dd, 2 H, H-5′), 2.35 (m, 2 H, H-4′, H-2a′), 1.70−2.11 (m, 3 H, H-2b′, *carba*-CH_2), 0.95−1.1 (m, 28 H, isopropyl). ^{13}C NMR (63 MHz, δ, $CDCl_3$): 156.5, 153.6, 139.9, 71.6, 62.2, 51.6, 50.1, 40.0, 27.6, 17.5, 17.3, 17.1, 13.4, 12.9. HRMS (DCI/NH_3): calcd for $C_{23}H_{42}O_3Si_2N5_5$ $(M + H)^+$ at *m*/*z* 492.2826, found 492.2832.

***N*^{6}-Benzoyl-2′-deoxyaristeromycin (6).** A solution of 2′-deoxy-3′,5′-*O*-TPDS-aristeromycin (**5**; 2.025 g, 4.1 mmol; dried three times by azeotroping with dry pyridine) in dry pyridine (20 mL) was cooled to 0 °C by means of an ice bath. To this solution was added benzoyl chloride (2.4 mL, 20.5 mmol), and the mixture was allowed to stand at room temperature for 3 h. The mixture was cooled again in an ice bath, and water (4 mL) was added followed by concentrated ammonia (8 mL). The resulting mixture was stirred for 40 min at 24 °C and then evaporated to dryness. The residue was dissolved in 60 mL of water, and the solution was extracted with ethyl acetate (3 × 30 mL), saturated sodium bicarbonate solution (30 mL), water (30 mL), and then brine (30 mL). The organic phase was dried over $MgSO_4$ and evaporated, giving an almost pure-yellow powder (2.95 g). The residue was treated with 1 M TBAF/THF solution (20 mL) overnight at 24 °C. The volatile materials were evaporated, and the residue was dissolved in ethyl acetate and applied to a silica gel column. The column was eluted with an increasing polarity gradient [ethyl acetate/methanol (10:1 < 5:1 < 3:1)]. The desired product **6** (1.05 g) was isolated as a yellow powder (72%), mp 165−166 °C. ^{1}H NMR (250 MHz, δ, $CDCl_3$): 8.88 (s, 1 H, H-8), 8.56 (s, 1 H, H-2), 8.19 (d, 2 H, Ph), 7.52 (m, 1 H, Ph), 7.39 (m, 2 H, Ph), 6.23 (brs, 1 H, NH), 5.42 (qt, 1 H, H-1′), 4.73 (m, 1 H, H-3′), 3.98 (m, 2 H, H-5′), 2.18−2.62 (2 × m + 2 × dt, 5 H, H-4′, H-2′, *carba*-CH_2). ^{13}C NMR (63 MHz, δ, $CDCl_3$): 167.4, 153.8, 151.77, 149.9, 149.04, 142.6, 135.8, 134.9, 132.34, 128.8, 126.5, 123.6, 123.3, 122.84, 72.9, 63.5, 54.2, 50.13, 41.37, 33.9. HRMS (DCI/NH_3): calcd for $C_{18}H_{20}O_3N_5$ $(M + H)^+$ at *m*/*z* 354.1566, found 354.1556.

5′-*O*-DMT-*N*^{6}-benzoyl-2′-deoxyaristeromycin (7). To a solution of *N*^{6}-benzoyl-2′-deoxyaristeromycin (**6**; 106 mg, 0.3 mmol; dried three times by coevaporation with dry pyridine) in dry pyridine (1.5 mL) were added DMT-Cl (recrystallized, 132 mg, 0.39 mmol) and DMAP (3.5 mg). The reaction was quenched after 4 h by pouring into ice-cold saturated sodium bicarbonate solution (10 mL). This mixture was extracted with ethyl acetate (3 × 5 mL), and the combined fractions were dried over $MgSO_4$. After partial evaporation, the solution was applied

to a silica gel column (15 g) and eluted with ethyl acetate/methanol/TEA (50:1:0.5 then 25:1:0.25). The fractions containing the pure material were collected and evaporated giving **7** (130 mg, 65% yield) as a pale-yellow solid, mp 95−97 °C. ^{1}H NMR (250 MHz, δ, $CDCl_3$): 8.70 (s, 1 H, H-8), 8.15 (d, 2 H, Ph), 7.91 (s, 1 H, H-2), 7.18−7.52 (m, 14 H, Ph), 6.79 (d, 2 H, Ph), 5.19 (qt, 1 H, H-1′), 4.42 (m, 1 H, H-3′), 3.76 (s, 6 H, 2 × OCH_3), 3.21−3.23 (m, 2 H, H-5′), 1.95−2.49 (2 × m + 2 × dt + m, 5 H, H-2′, H-4′, *carba*-CH_2). ^{13}C NMR (63 MHz, δ, $CDCl_3$): 169.2, 158.5, 153.5, 153.1, 150.5, 145.1, 142.3, 136.9, 134.2, 133.4, 132.9, 130.0, 128.8, 128.1, 127.9, 126.9, 113.18, 75.5, 65.1, 55.2, 53.7, 47.5, 40.1, 34.2. HRMS (FAB, NBA): calcd for $C_{39}H_{37}O_5$-Na $(M + Na)^+$ at *m/z* 678.2692, found 678.2678.

5′-*O*-DMT-*N*^{6}-benzoyl-2′-deoxyaristeromycin 3′-*O*-(2-Cyanoethyl *N*,*N*-diisopropylphosphoramidite) (8). To a solution of 5′-*O*-DMT-*N*^{6}-benzoyl-2′-deoxyaristeromycin (**7**; 125 mg, 0.19 mmol) in 2 mL of dry methylene chloride and 0.1 mL of dry triethylamine was added dropwise 0.085 mL of 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (0.38 mmol). The reaction mixture was stirred for 6 h and then diluted with 15 mL of dry toluene. The precipitated triethylamine hydrochloride was filtered under nitrogen, and the toluene was evaporated under reduced pressure. The residue **8** was used directly in the DNA synthesizer in dry acetonitrile solution. ^{1}H NMR (250 MHz, δ, $CDCl_3$): 8.72 (s, 1 H, H-8), 8.00 (d, 2 H, Ph), 7.98 (s, 1 H, H-2), 7.25−7.58 (m, 14 H, Ph), 6.79 (d, 2 H, Ph), 5.17 (qt, 1 H, H-1′), 4.56 (m, 1 H, H-3′), 3.77 (s, 6 H, OCH_3), 3.48 (m, 2 H, 2 × CHN), 3.27−3.31 (m, 2 H, H-5′), 2.10−2.49 (2 × m + 2 × dt, 5 H, H-2′, H-4′, *carba*-CH_2), 1.24 (d, 12 H, *isopropyl*). ^{31}P NMR (101 MHz, δ, $CDCl_3$): 149.4, 149.1.

Synthesis of Derivatives of 8-Oxo-7,8-dihydro-2′-deoxycarbaguanosine. Procedure A: Hydrazine Method. 2′-Deoxycarbaguanosine (12). A mixture of methanol (160 mL) and deionized water (80 mL) in a 500-mL flask was degassed with nitrogen for 30 min. To this solution was added 2-chloro-2′-deoxycarbainosine (**9**; 5.0 g) under nitrogen followed by anhydrous hydrazine (31 mL). The mixture was refluxed gently for 24 h, then solvent was removed in vacuo, and the residue was triturated with degassed 95% ethanol (200 mL). The resulting white precipitate was filtered under nitrogen to give 2-hydrazino-2′-deoxycarbainosine (**10**; 3.6 g). This compound readily decomposes upon standing and more rapidly on exposure to light. The subsequent reductive deamination was therefore carried out without further purification. In a hydrogenation flask was placed a 1:1 mixture of methanol and water (120 mL), and the mixture was degassed with nitrogen for 30 min. To this mixture was added wet Raney Ni (15 g), and the catalyst was saturated with H_2 for 1 h at 50 psi. The 2-hydrazino-2′-deoxycarbainosine (**10**; 3.6 g) dissolved in the same solvent mixture (120 mL, degassed) was added to the catalyst, and the mixture was hydrogenated for 24 h at 55 °C in a Parr apparatus at 50 psi. The reduction was monitored by HPLC analysis performed on a C-18 column using triethylammonium acetate buffer in acetonitrile in a linear gradient (0−10% acetonitrile over 50 min). After completion of the hydrogenation, the catalyst was removed using a filter aid and washed with a methanol−water (hot) mixture. The filtrate was concentrated to give the desired 2′-deoxycarbaguanosine (**12**; 2.8 g) as a white powder which was recrystallized from water, mp 243−245 °C dec (solubility: 7.1 g/100 g of water at 90 °C). ^{1}H NMR (300 MHz, δ, D_2O): 7.74 (s, 1 H, H-2), 4.72 (m, 1 H, H-1′), 4.15 (m, 1 H, H-3′), 3.51 and 3.60 (2 × dd, 2 H, H-5′), 2.36 (m, 1 H, H-4′), 1.58, 2.11, 2.36 (m, 4 H, H-2′, *carba*-CH_2). ^{13}C NMR (63 MHz, δ, D_2O): 157.2, 153.7, 151.4, 135.7, 71.9, 63.1, 52.5, 49.7, 41.0, 34.4. HRMS (DCI, NH_3): calcd for $C_{11}H_{16}N_5O_3$ $(M + H)^+$ at *m/z* 266.1253, found 266.1256.

Procedure B: Photolytic Method. 2-[[1-(2-Nitrophenyl)]ethyl]amino]-2′-deoxycarbainosine (11). To a solution of 2-chloro-2′-deoxycarbainosine (**9**; 300 mg, 1.06 mmol) in dry DMSO (10 mL) was added 2-(nitrophenyl)ethylamine (350 mg, 2.12 mmol; prepared from 2-nitroacetophenone by reductive amination), and the solution was placed in a sealed ampule. The reaction mixture was kept in an oil bath at 100 °C for 10 h in the dark. The DMSO was evaporated at 70 °C under reduced pressure, and the residue was triturated with water. After the liquid phase had been separated from the insoluble material, the oily residue was stirred with *tert*-butyl methyl ether followed by ethyl acetate/2-propanol (3:1). The insoluble residue was identified spectroscopically as the title compound **11**, ∼90% purity. ^{1}H NMR (300 MHz, δ, methanol-d_4): 7.3−7.7 (s + m, 5 H, H-2, *aromatic*), 5.31 (d, 1 H, NH), 4.68 (m, 1 H, H-1′), 4.10 (m, 1 H, H-3′), 3.86 (q, 1 H, $CHCH_3$), 3.59 (2 × dd, 2 H, H-5′), 2.36 (m, 1 H, H-4′), 1.58, 2.12, 2.36 (m, 4 H, H-2′, *carba*-CH_2), 1.15 (d, 3 H, CH_3). HRMS (DCI/NH_3): calcd for $C_{19}H_{23}N_6O_5$ $(M + H)^+$ at *m/z* 415.1730, found 415.1746.

Photolytic Debenzylation of 11. The oily nitrophenethyl derivative **11** was dissolved in a mixture of methanol (7 mL) and water (3 mL), and the solution was placed in a quartz cuvette and then photolyzed for 4.5 h between two clamp-held lamps at 360 nm. The progress of the reaction was monitored by UV spectrometry and TLC. After completion of the reaction, the solution was evaporated to dryness, and the residue was stirred with methanol. The insoluble product was removed by filtration and proved to be identical spectroscopically with the 2′-deoxycarbaguanosine (**12**) obtained by procedure A. The overall yield of **12**, mp 242−244 °C dec (145 mg), from 2-chloro-2′-deoxycarbainosine was 51%.

8-Bromo-2′-deoxycarbaguanosine (13). To a vigorously stirred suspension of 2′-deoxycarbaguanosine (**12**; 2.18 g, 8.23 mmol) in 15 mL of water was added portionwise 52 mL of bromine-saturated water (made by shaking 1.4 mL of Br_2 with 120 mL of water at room temperature) over 1.5 h. Stirring was continued at room temperature after the solution became homogeneous. At the end of 4 h, a solid began to separate from the reaction mixture. After 24 h, the thick solid was filtered and then crystallized from methanol to give 8-bromo-2′-deoxycarbaguanosine (**13**; 2.0 g). Evaporation to dryness of the mother liquor and crystallization of the residue twice from methanol yielded 0.55 g of a second crop, total yield 90%, mp 238−240 °C. ^{1}H NMR (300 MHz, δ, DMSO-d_6): 4.85 (dt, 1 H, H-1′), 4.01 (m, 1 H, H-3′), 3.27 and 3.46 (2 × dd, 2 H, H-5′), 2.40 (m, 1 H, H-4′), 1.66−1.95 (m, 4 H, H-2′, *carba*-CH_2). ^{13}C NMR (63 MHz, δ, D_2O): 155.7, 153.5, 152.4, 121.2, 117.3, 71.8, 63.4, 55.3, 49.7, 43.8, 38.1, 32.00. HRMS (EI): calcd for $C_{11}H_{14}N_5O_3Br$ (M^+) at *m/z* 343.0280, found 343.0294.

8-(Benzyloxy)-2′-deoxycarbaguanosine (14). A solution of 8-bromo-2′-deoxycarbaguanosine (**13**; 2.5 g, 7.29 mmol) in dry dimethyl sulfoxide (11 mL) was added to a solution of sodium benzyl oxide (prepared from 19.4 mL of benzyl alcohol and 610 mg of sodium heated at 80 °C for 2 h) under nitrogen. The heavy syrup was stirred for 24 h at 65 °C and then allowed to cool to room temperature. After neutralization with glacial acetic acid, the bulk of the dimethyl sulfoxide was removed by vacuum distillation at 100 °C (bath temperature). The remaining liquid was then poured into *tert*-butyl methyl ether (500 mL) with stirring. The ether layer was decanted thereby removing the excess benzyl alcohol. TLC analysis of the residue showed two new substances in addition to unreacted starting material. The new materials were purified by column chromatography using methylene chloride/methanol (4:1) as the eluant. The desired compound **14** was isolated as an oil in 46% yield (1.24 g). ^{1}H NMR (300 MHz, δ, MeOH-d_4): 7.30−7.50 (d, 5 H, *aromatic*), 5.47 (s, 1 H, *benzylic* CH_2), 4.83 (m, 1 H, H-1′), 3.97 (m, 1 H, H-3′), 3.35 and 3.45 (2 × dd, 2 H, H-5′), 2.23 (m, 1 H, H-4′), 1.77−2.05 (m, 4 H, H-2′, *carba*-CH_2). ^{13}C NMR (63 MHz, δ, MeOH-d_4): 160.1, 155.1, 154.1, 153.1, 137.1, 130.0, 129.9, 112.3, 74.3, 73.3, 65.0, 52.7, 51.1, 40.8, 39.5, 33.3. HRMS (EI): calcd for $C_{18}H_{21}N_5O_4$ (M^+) at *m/z* 371.1594, found 371.1597.

The second product was identified as 8-oxo-2′-deoxycarbaguanosine (0.6 g, 29%). ^{1}H NMR (300 MHz, δ, MeOH-d_4): 4.78 (m, 1 H, H-1′), 4.05 (m, 1 H, H-3′), 3.45 (t, 2 H, H-5′), 2.25 (m, 1 H, H-4′), 1.82−2.1 (m, 4 H, H-2′, *carba*-CH_2). FAB/MS (+ve ion, thioglycerol matrix): *m/z* 282 $(M + H)^+$.

***N*²-Isobutyryl-8-(benzyloxy)-2′-deoxycarbaguanosine (15).** To a suspension of 8-(benzyloxy)-2′-deoxycarbaguanosine (**14**; 280 mg, 0.75 mmol; dried by evaporating three times with pyridine) in 5 mL of dry pyridine was added trimethylchlorosilane (0.63 mL, 3.90 mmol), and the reaction mixture was stirred for 30 min. To the clear solution was added isobutyric anhydride (0.81 mL, 3.75 mmol), and the mixture was then stirred overnight at room temperature. The solution was cooled to 0 °C in an ice bath, and water (2 mL) was added followed by the addition of concentrated ammonia (1 mL) at the same temperature. The solution was stirred for 30 min while the temperature warmed to ambient and was then evaporated to dryness. The residue was dissolved in methanol and mixed with a small portion of silica gel. The solvent was removed by evaporation, and the dried residue was applied to a silica gel column and eluted with a linear gradient of a methylene chloride/methanol mixture (20:1 up to 15:2). The main fractions yielded **15** as a white powder in 60% yield (200 mg). ^{1}H NMR (300 MHz, δ, MeOH-d_4): 7.37−7.52 (ddm, 5 H, *aromatic*), 5.47 (s, 1 H, *benzylic* CH_2), 5.05 (m, 1 H, H-1′), 4.19 (m, 1 H, H-3′), 3.52 and 3.61 (2 × dd, 2 H, H-5′), 2.71 (2 × t, 1 H, *isobutyryl CH*), 2.55 (m, 1 H, H-4′), 1.92−2.20 (m, 4 H, H-2′, *carba*-CH_2), 1.24 (2 × s, 2 CH_3). ^{13}C NMR (63 MHz, δ, MeOH-d_4): 168.5, 157.0, 155.5, 150.5, 147.6, 129.8, 129.7, 117.5, 118.7, 73.6, 73.3, 64.4, 52.9, 50.7, 39.3, 33.0, 19.4, 19.3. HRMS (DCI, NH_3): calcd for $C_{22}H_{28}N_5O_5$ $(M + H)^+$ at *m*/*z* 442.2090, found 442.2093.

***N*²-Isobutyryl-8-oxo-7,8-dihydro-2′-deoxycarbaguanosine (16).** A solution of *N*²-isobutyryl-8-(benzyloxy)-2′-deoxycarbaguanosine (**15**; 200 mg, 0.45 mmol) in methanol (8 mL) was hydrogenated at room temperature in the presence of 10% Pd/C catalyst (80 mg) for 24 h. The reaction mixture was then filtered through a Celite bed, and the filtrate was concentrated in vacuo. The product (148 mg) was used in the next step without purification. ^{1}H NMR (300 MHz, δ, MeOH-d_4): 4.90 (m, 1 H, H-1′), 4.21 (m, 1 H, H-3′), 3.66 and 3.56 (2 × m, 2 H, H-5′), 2.63 (2 × t, 1 H, *isobutyryl CH*), 2.50 (m, 1 H, H-4′), 1.82−2.05 (m, H-2′, 4 H, *carba*-CH_2), 1.12 (2 × s, 2 CH_3). ^{13}C NMR (63 MHz, δ, MeOH-d_4): 181.3, 168.0, 154.0, 148.4, 147.6, 104.3, 73.4, 64.1, 51.2, 50.5, 38.2, 36.9, 31.8, 19.0. HRMS (EI): calcd for $C_{15}H_{21}N_5O_5$ (M^+) at *m*/*z* 351.1543, found 351.1546.

5′-*O*-DMT-*N*²-isobutyryl-8-oxo-7,8-dihydro-2′-deoxycarbaguanosine (17). *N*²-Isobutyryl-8-oxo-7,8-dihydro-2′-deoxycarbaguanosine (**16**; 120 mg, 0.34 mmol) was dried with pyridine (3 × 5 mL). The dry material was suspended in pyridine (2 mL) containing DMT-Cl (0.127 mg, 0.37 mmol). After stirring for 1 h at room temperature, the reaction was quenched by pouring into an ice-cold saturated solution of sodium bicarbonate (5 mL). The solution was extracted with methylene chloride (3 × 5 mL), and the combined fractions were dried over $MgSO_4$. After partial evaporation the solution was applied to a silica gel column which was then eluted with methylene chloride/methanol/triethylamine (20:1:0.5). The main fractions yielded pure **17** (200 mg), mp 164−165 °C. ^{1}H NMR (300 MHz, δ, MeOH-d_4): 7.03−7.74 (m, 11 H, Ph), 6.72 (d, 2 H, Ph), 4.95 (m, 1 H, H-1′), 4.17 (m, 1 H, H-3′), 3.66 (2 × s, 6 H, 2 × OCH_3), 3.23 and 3.05 (2 × m, 2 H, H-5′), 2.55 (m, 2 H, *isobutyryl CH*, H-4′), 1.84−2.18 (m, 4 H, H-2′, *carba*-CH_2), 1.08 (2 × s, 6 H, 2 CH_3). ^{13}C NMR (63 MHz, δ, MeOH-d_4): 182.2, 160.4, 154.7, 152.0, 149.2, 148.3, 147.1, 138.0, 131.6, 129.7, 129.0, 128.0, 114.3, 105.0, 97.9, 87.5, 74.6, 66.6, 56.1, 38.7, 37.1, 34.0, 19.7. HRMS (FAB/NBA): calcd for $C_{36}H_{39}N_5O_7Na$ $(M + Na)^+$ at *m*/*z* 676.2744, found 676.2747.

5′-*O*-DMT-*N*²-isobutyryl-8-oxo-2′-deoxycarbaguanosine 3′-*O*-(2-Cyanoethyl *N*,*N*-diisopropylphosphoramidite) (18). A modification of the procedure used by Bodepudi et al. (*23*) was used. A thoroughly dried sample of 5′-*O*-DMT-*N*²-isobutyryl-8-oxo-7,8-dihydro-2′-deoxycarbaguanosine (0.13 g, 0.2 mmol) was dissolved in a mixture of dry tetrahydrofuran (1 mL) and dry methylene chloride (1 mL) under N_2 at room temperature. To this solution were added dry triethylamine (0.085 mL) and 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (0.105 mL, 0.22 mmol). The mixture was stirred at 24 °C for 2 h during which time a white precipitate of Et_3N·HCl separated. A second portion (0.035 mL) of the phosphoramidite reagent was added to complete the conversion. After a further 1 h, the mixture was filtered and the collected solid was washed with toluene (2 mL). The filtrate was concentrated. The residual glassy solid **18** (0.175 g) showed two single peaks in the ^{31}P NMR spectrum as noted below for the expected diastereoisomers with virtually no other absorption in the spectrum. The material was judged pure enough to be used directly in DNA synthesis. ^{1}H NMR (300 MHz, δ, MeOH-d_4): 7.03−7.74 (m, 11 H, Ph), 6.72 (d, 2 H, Ph), 5.03 (m, 1 H, H-1′), 4.18 (m, 1 H, H-3′), 3.76 (s, 6 H, 2 × OCH_3), 3.44, 3.54, 3.56 (2 × m, 4 H, H-5′, 2 × CHN), 2.64−2.76 (m, 2 H, *isobutyryl CH*, H-4′), 1.84−2.18 (m, 4 H, H-2′, *carba*-CH_2), 1.26 (d, 12 H, *isopropyl*), 1.05 (2 × s, 2 CH_3). ^{31}P NMR (101 MHz, δ, $CDCl_3$): 147.6, 147.9.

8-Bromo-2′-deoxyaristeromycin (20). 2′-Deoxyaristeromycin (**19**; 0.5 g) was dissolved in sodium acetate buffer (60 mL, 1 M, pH 4) which was then treated with bromine (150 mL) in the same buffer (30 mL). The reaction mixture was stirred for 5 h in the dark, and the solution was decolorized with a small amount of $NaHSO_3$ solution and then saturated with sodium chloride. The solution was then extracted with chloroform (4 × 5 mL). The organic extracts were dried over $MgSO_4$, filtered, and evaporated to give **20** (665 mg) of better than 95% purity. This was used directly in the next step. A small portion was recrystallized from ethanol/hexane to give the analytically pure material. ^{1}H NMR (250 MHz, δ, DMSO-d_6): 7.80 (s, 1 H, H-2), 7.35 (brs, 2 H, NH_2), 5.08 (m, 1 H, H-1′), 4.63 (m, 1 H, H-3′), 4.17 and 3.65 (2 × dd, 2 H, H-5′), 2.75 (m, 1 H, H-4′), 1.95−2.24 (m, 4 H, H-2′, *carba*-CH_2). ^{13}C NMR (63 MHz, δ, DMSO-d_6): 175.1, 155.7, 152.8, 151.42, 126.6, 120.9, 73.6, 64.2, 50.6, 48.3, 38.7, 31.8, 20.5. HRMS (EI): calcd for $C_{11}H_{14}O_2N_5Br$ (M^+) at *m*/*z* 327.0331, found 327.0329.

8-(Benzyloxy)-2′-deoxyaristeromycin (21). To dry benzyl alcohol (15 mL) at 65 °C in a 100-mL flask was added sodium (408 mg, 17.7 mmol) in small pieces (weighed and cut under dry pentane). The temperature was raised to 80 °C, and the metal dissolved within 2 h. 8-Bromo-2′-deoxyaristeromycin (**20**; 1.02 g, 3.2 mmol) was added, and the reaction mixture was heated for a further 10 h at the same temperature and monitored by TLC [methylene chloride/methanol (5:1), long plate]. The benzyl alcohol was then removed under reduced pressure, and the residue was triturated with *tert*-butyl methyl ether giving crude **21** (630 mg) as a brown precipitate. This was purified through a short silica gel column using ethyl acetate/methanol (7.5:1) as the mobile phase. This yielded pure **21** (510 mg) as a faintly yellow powder. ^{1}H NMR (300 MHz, δ, MeOH-d_4): 8.05 (s, 1 H, H-2), 7.50 (d, 2 H, *aromatic*), 7.37 (m, 2 H, *aromatic*), 7.30 (dd, 1 H, *aromatic*), 5.53 (s, 1 H, *benzylic* CH_2), 5.12 (m, 1 H, H-1′), 4.17 (m, 1 H, H-3′), 3.63 and 3.49 (2 × dd, 2 H, H-5′), 2.53 (m, 1 H, H-4′), 1.96−2.24 (m, 4 H, H-2′, *carba*-CH_2). ^{13}C NMR (63 MHz, δ, MeOH-d_4): 156.2, 155.2, 151.7, 150.7, 136.8, 130.03, 129.97, 129.7, 116.42, 74.2, 73.45, 67.0, 52.9, 50.9, 39.48, 33.25. HRMS (FAB): calcd for $C_{18}H_{22}O_3N_5$ $(M + H)^+$ at *m*/*z* 356.1723, found 356.1727.

8-Oxo-7,8-dihydro-2′-deoxyaristeromycin (22). A sample of purified 8-(benzyloxy)-2′-deoxyaristeromycin (**21**; 160 mg) was hydrogenated at 50 °C and 50 psi in methanol (7 mL) using a 10% Pd/C catalyst (35 mg). After 3 h, TLC analysis [solvents, ethyl acetate/methanol (3:1)] showed complete conversion to product. The reaction mixture was filtered through a Celite bed and evaporated to dryness. This gave an almost quantitative yield (117 mg) of **22** which was used directly to prepare **23**. ^{1}H NMR (300 MHz, δ, MeOH-d_4/D_2O): 7.95 (s, 1 H, H-2), 5.07 (m, 1 H, H-1′), 4.19 (m, 1 H, H-3′), 3.66 and 3.50 (2 × dd, 2 H, H-5′), 2.54 (m, 1 H, H-4′), 184−2.19 (m, 4 H, H-2′, *carba*-CH_2). ^{13}C NMR (63 MHz, δ, MeOH-d_4/D_2O): 155.1, 152.4, 149.3, 149.0, 105.8, 74.7, 65.4, 52.0, 51.2, 38.8, 32.7. HRMS (DCI/NH_3): calcd for $C_{11}H_{16}N_5O_3$ $(M + H)^+$ at *m*/*z* 266.1253, found 266.1256.

8-Oxo-2′-deoxy-7,8-dihydrocarbainosine (23). A dilute solution of 8-oxo-2′-deoxyaristeromycin (**22**) was prepared (110 mg in 750 mL of deionized water) and treated with adenosine

deaminase (30 mg, type II, purified, calf intestine; Sigma). After 8 days the enzymatic deamination was complete according to TLC analysis [methylene chloride/methanol/ammonium hydroxide (30:10:2)]. The solution was evaporated to one-third its volume, and the title compound precipitated out. The solid was isolated by centrifugation, washed with cold water, and dried, yielding 95 mg (86%) of essentially pure **23**. Because this compound has a very low solubility in the generally employed solvents, the crude product was again used in the next step without further purification. ^{1}H NMR (300 MHz, δ, pyridine-d_5/D_2O): 7.66 (s, 1 H, H-2), 4.56 (m, 1 H, H-1′), 4.07 (m, 1 H, H-3′), 3.55 and 3.42 (2 × dd, 2 H, H-5′), 2.22 (m, 1 H, H-4′), 1.84−1.94 (m, 4 H, H-2′, *carba*-CH_2). ^{13}C NMR (63 MHz, δ, pyridine-d_5/D_2O): 152.9, 148.1, 145.5, 145.3, 108.1, 72.9, 63.4, 50.3, 48.8, 37.1, 31.3. HRMS (EI): calcd for $C_{11}H_{14}N_4O_4$ (M^+) at *m*/*z* 266.1015, found 266.1023.

5′-*O*-DMT-8-oxo-7,8-dihydro-2′-deoxycarbainosine (24). To a suspension of 8-oxo-7,8-dihydro-2′-deoxycarbainosine (**23**; 95 mg, 0.36 mmol) in dry pyridine (1.5 mL) was added 4,4′-DMT-Cl (134 mg, 0.39 mmol) in one portion. The reddish suspension was stirred overnight which resulted in a clear solution. The reaction was monitored by TLC using methylene chloride/methanol (2:1) as the eluant. The mixture was then cooled in an ice bath, quenched with water (5 mL), and extracted with methylene chloride (3 × 5 mL). The combined organic extracts were washed with water (2 × 4 mL) and brine (4 mL) and then dried over $MgSO_4$. After evaporation of the solvent, the crude product was purified on a silica gel column using methylene chloride/methanol (20:1) containing 2% triethylamine as the eluting solvent mixture. The desired compound **23** was obtained in 67% yield (140 mg). ^{1}H NMR (300 MHz, δ, pyridine-d_5): 8.02 (s, 1 H, H-2), 7.23−7.64 (m, 11 H, Ph), 6.77 (d, 2 H, Ph), 5.52 (m, 1 H, H-1′), 4.69 (m, 1 H, H-3′), 3.47 (s, 6 H, 2 × OCH_3), 3.38 and 3.65 (2 × dd, 2 H, H-5′), 2.90 (m, 1 H, H-4′), 2.22−2.55 (2 × m + 2 × dt, 4 H, H-2′, *carba*-CH_2). ^{13}C NMR (63 MHz, δ, pyridine-d_5): 158.5, 153.1, 151.8, 146.1, 145.9, 143.8, 136.7, 130.4, 128.4, 127.8, 126.1, 113.2, 109.4, 99.5, 85.8, 73.9, 65.5, 54.7, 50.2, 36.5, 32.6. HRMS (FAB/NBA): calcd for $C_{32}H_{32}N_4O_6Na$ (M + Na^+) at *m*/*z* 591.2220, found 591.2212.

5′-*O*-DMT-8-oxo-7,8-dihydro-2′-deoxycarbainosine 3′-*O*-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite) (25). A thoroughly dried portion of 5′-*O*-DMT-8-oxo-7,8-dihydro-2′-deoxycarbainosine (**24**; 85 mg, 0.15 mmol) was dissolved in dry tetrahydrofuran (0.8 mL) and dry methylene chloride (0.8 mL) under nitrogen. To this solution were added dry triethylamine (0.064 mL) and 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.079 mL, 0.17 mmol). The reaction mixture was strirred for 2 h at room temperature, and a white precipitate separated (Et_3N·HCl). After 2 h, a second portion of phosphoramidite reagent (0.026 mL) was added to the reaction mixture to achieve total conversion. After the mixture had been stirred for a further 1 h, dry toluene (10 mL) was added to complete the precipitation of triethylamine hydrochloride. The precipitate was filtered under nitrogen and washed with dry toluene (2 mL), and the filtrate was concentrated. The title compound **25**, obtained as a glassy solid (115 mg), was pure enough to use directly for DNA synthesis as confirmed by ^{31}P NMR analysis. ^{1}H NMR (250 MHz, δ, $CDCl_3$): 7.94 (s, 1 H, H-2), 7.25−7.49 (m, 14 H, Ph), 6.80 (d, 2 H, Ph), 5.11 (m, 1 H, H-1′), 4.33 (m, 1 H, H-3′), 3.76 (s, 6 H, 2 × OCH_3), 3.51 (m, 2 H, 2 × CHN), 3.32−3.42 (m, 2 H, H-5′), 2.72 (m, 1 H, H-4′), 2.05−2.49 (2 × m + 2 × dt, 4 H, H-2′, *carba*-CH_2), 1.24 (d, 12 H, *isopropyl*). ^{31}P NMR (101 MHz, δ, $CDCl_3$): 149.3, 148.9.

Synthesis of DNA Oligomers. The examples described below illustrate the general methods employed for the synthesis of all of the oligomers described in this and a related paper (*17*). Oligomers were synthesized on an ABI (Foster City, CA) 394 DNA synthesizer using standard phosphoramidite protocols. The selected modified phosphoramidite was introduced into several oligomers. After each synthesis, the product was deprotected by incubation in 1 mL of 28% ammonium hydroxide, 16 h at 55 °C. The solution was evaporated to dryness (Savant Instruments, Inc., Farmingdale, NY), and the crude product was resuspended in water. Purification was accomplished on a Waters Chromatography (Milford, MA) system equipped with a 990 photodiode array detector. For DMT-protected DNA, a μBondapak C_{18} column was employed using a gradient of 16−35% acetonitrile and triethylammonium acetate buffer (0.1 M, pH 7.2) in 30 min. Collections were pooled and evaporated to dryness (Savant Instruments, Inc., Farmingdale, NY), and the crude product was resuspended in water.

A. 5′-TT(dAris)TT-3′. This was prepared as described above using **8**. The DMT oligomer was found to have a retention time of 20.4 min using the conditions outlined above. The collected material was dried and treated with 80% acetic acid (0.2 mL) for 30 min. The resulting solution was dried and rechromatographed by HPLC. A single peak was obtained with a retention time of 16.56 min using 0−15% acetonitrile in 0.1 M triethylammonium acetate over 30 min. The collected portion was submitted for mass analysis. MS (FAB, Gly): calcd 1465.3 Da (M − H^-), found *m*/*z* 1464.

B. 5′-CTCTCCCTTC(dAris)CTCCTTTCCTCT-3′. The method of synthesis again followed the procedure described above. The DMT oligomer was purified and found to have a retention time of 21.5 min on the C_{18} column. The DMT group was removed with 80% acetic acid (room temperature, 17.5 min) and resulted in a single substance when rechromatographed. This was collected and submitted for mass analysis. MS (ESI): calcd 6761.4 Da, found 6760.8 Da.

C. 5′-CTCTCCCTTC(8-oxo-carba-dG)G-CTCCTTTCCT-CT-3′. This was prepared according to the procedure described above using **18**. β-Mercaptoethanol (7 μL, 0.1 N) was added to the ammonia solution to avoid any oxidation of the modified base. The DMT oligomer was found to have a retention time of 18.4 min using the conditions outlined above. The collected material was dried and treated with 80% acetic acid (0.2 mL) for 30 min. The resulting solution was then dried and rechromatographed by HPLC. A single peak was obtained with a retention time of 23.5 min using a linear gradient of 0−15% acetonitrile in 0.1 M triethylammonium acetate over 30 min. The UV spectrum was consistent with an oligomer containing an 8-oxo-dG moiety with a peak at 266 nm and a broad shoulder from 292 to 310 nm. The collected portion was submitted for mass analysis. MS (ESI): calcd 6793.3 Da, found 6793.7 Da.

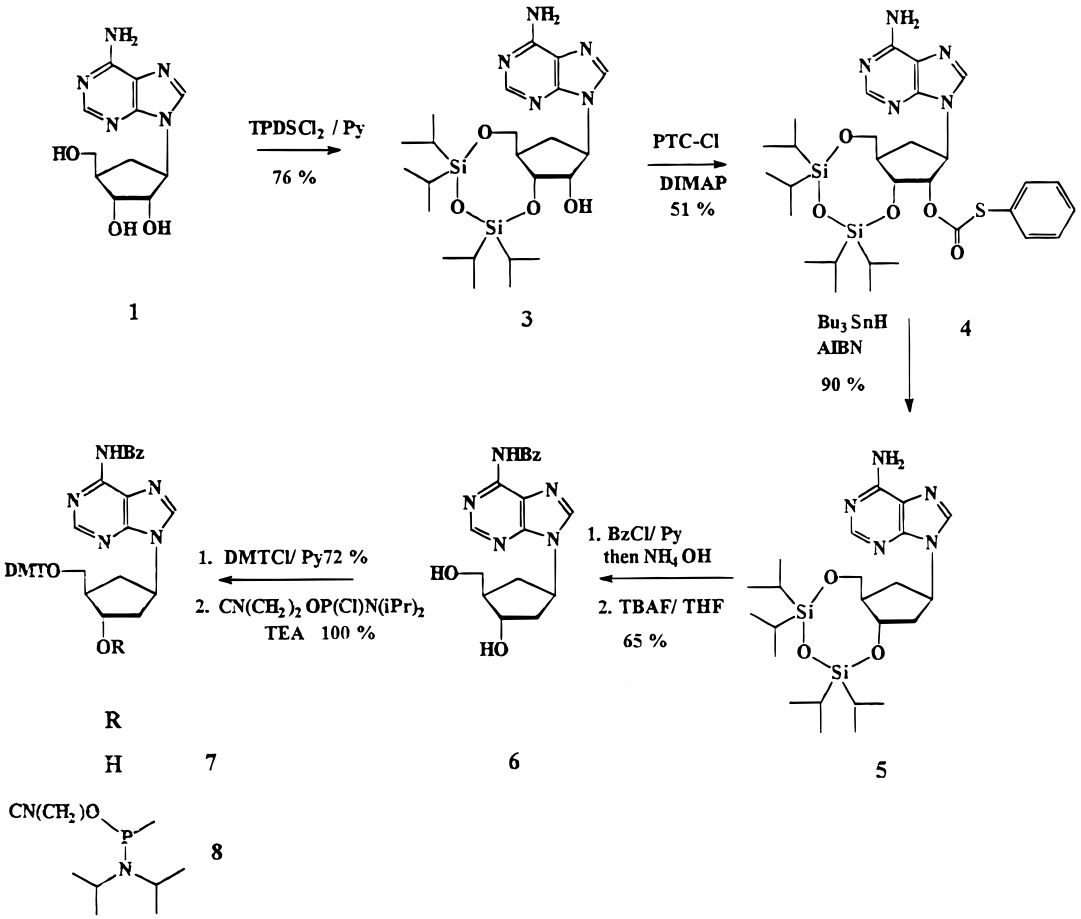
D. 5′-TTCAGTCA(8-oxo-carba-dI)TCAGTCGTA-3′. This was prepared as described above using **25**. β-Mercaptoethanol (7 μL, 0.1 N) was added to the ammonia solution to avoid further oxidation of the modified base. The DMT oligomer was found to have a retention time of 27.5 min using the conditions outlined above. The collected material was dried and treated with 80% acetic acid (0.2 mL) for 30 min. The resulting solution was evaporated to dryness and rechromatographed by HPLC. A single peak was obtained with a retention time of 36.3 min using a linear gradient of 0−15% acetonitrile in 0.1 M triethylammonium acetate over 30 min. The UV spectrum showed a maximum at 260 nm and was consistent with an oligomer previously synthesized that contained the analogous nucleoside residue 8-oxo-dI. The collected portion was submitted for mass analysis. MS (ESI): calcd 5488.6 Da, found 5488.1 Da.

Results and Discussion

In our approach to the study of these two enzymes (MutM and MutY), we now report the synthesis and incorporation into oligomeric DNA of the carbocyclic analogues of the 2-deoxynucleosides discussed above, namely, 2′-deoxyaristeromycin, 8-oxo-7,8-dihydro-2′-deoxycarbaguanosine, and 8-oxo-7,8-dihydro-2′-deoxycarbainosine. The oligomers are useful (1) for the preparation of affinity chromatography substrates for enzyme isolation and (2) for the characterization of, and mechanism studies on, the DNA-repair processes.

Synthesis and Incorporation of dAris into DNA. Our initial synthesis started from aristeromycin (**1**) and

Scheme 2

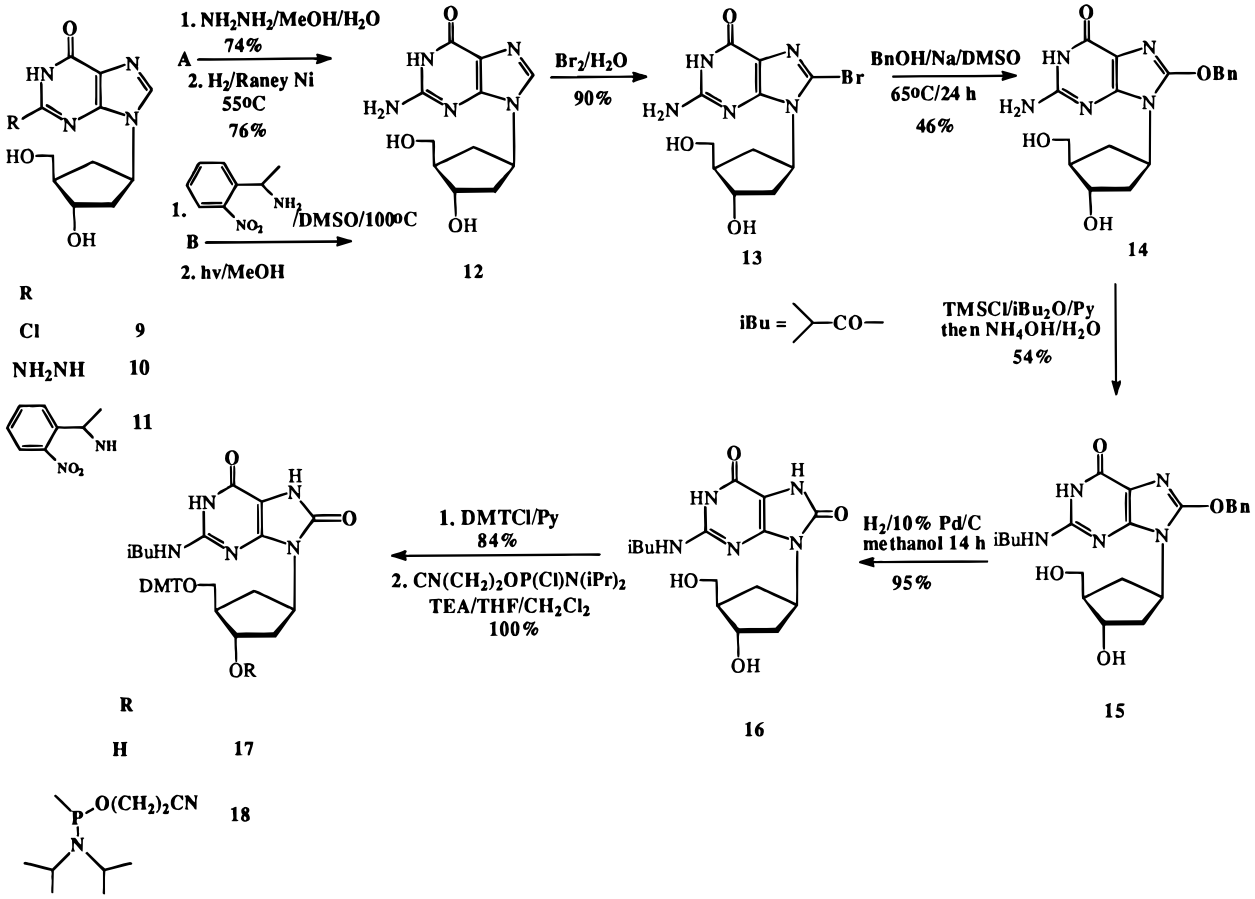


followed the Markiewicz−Robins deoxygenation (*18*) route described for 2-deoxyadenosine, rather than the original procedures used by Marumoto (*19*). General phosphoramidite chemistry was used to incorporate dAris into DNA oligomers using the standard protecting groups, namely, 5′-*O*-DMT and N^6-benzoyl (Scheme 2). The coupling efficiency was comparable to that of the natural base during the synthesis of a 23-mer [5′-CTC TCC CTT C(dAris)C TCC TTT CCT CT-3′]. The oligomers were then characterized by electrospray mass spectrometry. In simple binding experiments (*20*), the duplex containing dAris opposite to dG was recognized but not cleaved by the MutY enzyme as might be anticipated from the postulated mechanism of cleavage (S_N2 displacement).

Synthesis of 8-Oxo-7,8-dihydro-2′-deoxycarbaguanosine and Incorporation into DNA. The synthesis of this compound began with 2-chloro-2′-deoxycarbainosine (**9**; Scheme 3), which can be obtained from aristeromycin (**1**) in several steps (*21*). In our first approach to convert this compound to carba-dG (**12**), we attempted a direct amination by using ammonia under pressure, but after several hours mainly starting material was recovered. In a second attempt, the N^2-benzyl derivative of carba-dG was prepared from **9** in 51% yield, but the subsequent attempt to effect debenzylation by hydrogenation at 50 psi and 60 °C was not successful. Photochemical benzylic cleavage, however, readily yielded carba-dG (**12**) when the 2-nitrobenzyl analogue **11** was employed. The latter was easily prepared by treating **9** with an excess of 2-(nitrophenyl)ethylamine (*22*) in dimethyl sulfoxide at 100 °C for 12 h in the dark. On a small scale the photolytic removal of the 2-nitrophenethyl group was conducted at room temperature with lamps radiating at ∼360-nm wavelength, and this readily provided the desired carbocyclic nucleoside (*23*). However, on a larger scale, we simply followed the Long−Robins−Townsend methodology (*24*) for the ribonucleoside series and converted 2-chloro-2′-deoxycarbainosine (**9**) into the corresponding 2-hydrazino derivative **10** in 73% yield. Subsequent hydrogenation of this oxygen-sensitive compound at 50 °C and 50 psi for 20 h followed by crystallization of the product from water gave very pure carba-dG (**12**) in 72% yield.

The synthesis of the protected 8-oxo-carba-dG (**17**) was carried out in five steps using a modification of Bodepudi's procedure (*25*) for 8-oxo-dG (**2**). However, the initial bromination to give **13**, using aqueous bromine at room temperature for 5 h, was considerably slower than in the case of the 2′-deoxyribose analogue. This suggests either that in the carbocyclic analogues, the 7,8-double bond of the purine ring has a considerably reduced electron density or that in the natural nucleoside, the ring oxygen can assist the process, perhaps through bromine complexation. The replacement of the 8-bromo group by benzyloxy was then accomplished in 46% yield using sodium benzyl oxide and DMSO at 70 °C for 24 h. This reaction was not clean and afforded, besides **14**, some recovered starting material and a 29% yield of 8-oxo-carba-dG. Nevertheless, **14** was readily separated by chromatography. Surprisingly, it did not crystallize as readily as the analogous compound derived from the natural nucleoside, and the benzyl group showed an increased tendency to be cleaved under acidic conditions.

Scheme 3



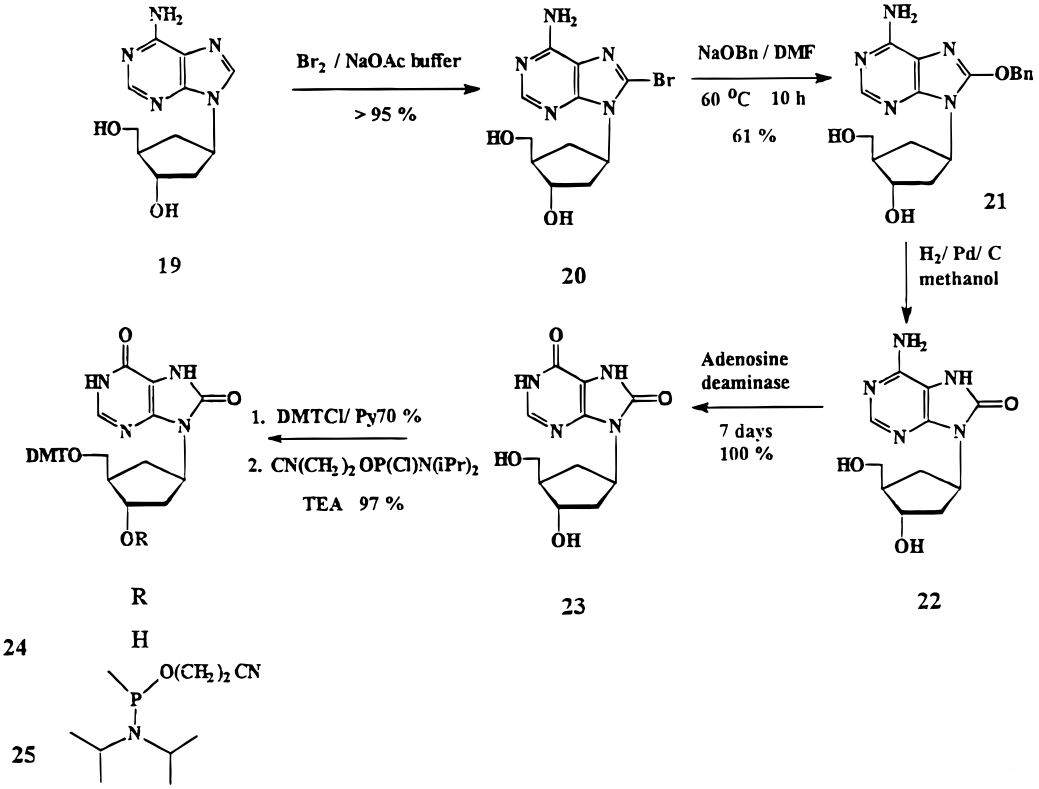
Protection of the N^2-amino group of **14** by the 'transient protection' method (*26*) to give **15** was best carried out at this stage. If the benzyl group was removed first, the subsequent acylation led to undesired side products. The N^2-protected 8-benzyl derivative **15** was then hydrogenated over 10% Pd/C at room temperature and gave N^2-isobutyryl-8-oxo-carba-dG (**16**). The DMT protection of the 5-hydroxyl to give **17** was carried out by the standard procedure (DMT-Cl/Py), but the reaction was considerably slower (12 h) than that in the deoxyribose series (15 min). Attempts to catalyze the process by DMAP led only to increased formation of the 3′,5′-bis-*O*-DMT derivative. The preparation of the phosphoramidite **18** was carried out by the 'nonaqueous workup technique' developed by Bodepudi (*27*) in our laboratory, and again the reaction proved to be slower than that of the deoxyribose analogue requiring both a much longer reaction period and a larger excess of reagent.

The use of **18** to incorporate 8-oxo-carba-dG residues into DNA [pentamer, TT(8-oxo-carba-dG)TT; 23-mer, 5′-CTC TCC CTT C(8-oxo-carba-dG)C TCC TTT CCT CT-3′] was carried out by automated synthesis and proceeded with excellent coupling efficiency (>95%). Deprotection and isolation of these oligomers followed the normal procedures used for oligomers containing 8-oxo-dG residues (*25*). In preliminary experiments it was found that MutM binds to a 23-mer duplex containing *8*-oxo-carba-dG opposite to dC in the sequence indicated above but that cleavage of the glycosidic bond is totally inhibited (K_d = 15−25 nM). The detailed study has been published elsewhere (*20, 28*).

Synthesis of 8-Oxo-7,8-dihydro-2′-deoxyaristeromycin and 8-Oxo-7,8-dihydro-2′-deoxycarbainosine. In previous studies with MutM enzyme (*29*) (FaPy protein), it was found that 8-oxo-2′-deoxyinosine had an enzyme binding affinity similar to that of 8-oxo-dG. Therefore, it appeared that the carbocylic analogue might serve as a good nonenzymatically cleavable substrate for biochemical studies and for enzyme purification. The synthesis from 2′-deoxyaristeromycin (**19**) is reasonably short (Scheme 4). Bromination of **19**, obtained by debenzoylation of **6**, was carried out according to Ettner (*30*), but an extractive workup, rather than direct crystallization, was used to obtain 8-bromo-dAris (**20**) of acceptable purity in 65% yield. This was used directly in the benzylation step, which afforded **21** in 61% yield. Hydrogenation of **21** then removed the benzyl group in excellent yield. Chemical deamination of 8-oxo-dAris (**22**, 8-oxo-7,8-dihydro-2′-deoxycarbaadenosine) did not proceed well, whereas deamination prior to reductive debenzylation (i.e., of **21**) led to partial loss of the benzyl group, thus making purification of the product difficult. Enzymatic deamination proved more successful. Adenosine deaminase is known to have a low substrate specificity (*31, 32*) for purine nucleosides, having substituents at the 8-position. Thus, when **22** was treated with this enzyme for 7 days, the desired 8-oxo-7-dehydro-2′-deoxycarbainosine (**23**) was obtained in quantitative yield. The formation of the DMT-phosphoramidite (**25**) and subsequent incorporation into oligomeric DNA were accomplished exactly as described above for 8-oxo-carba-dG.

Stability of 8-Oxo-carba-dG toward Aerobic Oxidation. The deprotection step during the synthesis of oligomeric DNA requires ammonia treatment at 55 °C for 5−15 h. When 8-oxo-dG residues are present, exten-

Scheme 4



sive degradation (*33*, *34*) and cleavage of the DNA occur if an antioxidant such as mercaptoethanol or ascorbic acid is not added (*35*, *36*). Although no cleavage of DNA containing 8-oxo-carba-dG residues occurs during this step, there is substantial degradation of the heterocyclic base, and an antioxidant must also be included in this case. Further work on the nature of this oxidation will be the subject of a future publication.

Acknowledgment. We are very grateful to Drs. Arthur P. Grollman, Kenneth Breslauer, Craig Gelfand, and Carlos de los Santos for their interest in these studies. This research was supported by a grant (CA 4799506) from the National Cancer Institute of the National Institutes of Health.

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TX9701539