Amidine Protecting Groups for Oligonucleotide Synthesis[1]

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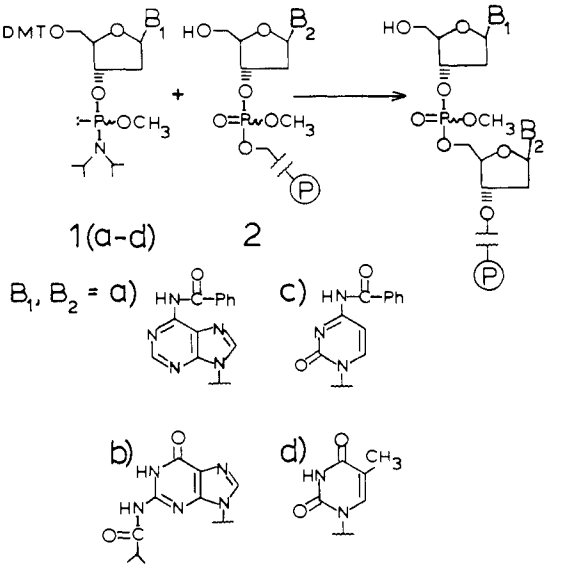
Abstract: Amidines have been investigated for protection of deoxyadenosine, deoxycytidine, 5-methyldeoxycytidine, cytidine, and deoxyguanosine. These amidine-protected nucleosides and their 5′-bis(*p*-anisylphenyl)methyl derivatives were prepared in high yield (79–100%) by simple one-flask procedures, converted to their respective phosphoramidites [3′-*O*-((diisopropylamino)methoxyphosphino)] with bis(diisopropylamino)methoxyphosphine, and used in situ for synthesizing d(G-G-G-A-A-T-T-C-C-C). This deoxyoligonucleotide was recognized by *Eco*RI restriction endonuclease, suggesting that amidines can be used to generate biochemically reactive DNA. Deprotection can be routinely performed by using concentrated aqueous ammonia. However, either ethylenediamine/phenol/water or aqueous ammonia containing ammonium acetate enhances amidine deprotection rates severalfold. In addition to their intrinsic ease of preparation, amidines were shown to have certain advantages when compared to amide-protected nucleosides. (1) Deoxyadenosine when protected with amidines was approximately 20-fold more resistant to depurination than 6-*N*-benzoyldeoxyadenosine under certain acidic conditions used for synthesizing DNA. (2) Unlike amide-protected cytidine and deoxycytidine, amidine derivatives of this nucleoside are not susceptible to attack on C-4 by primary amines. Thus these amines can be used in conjunction with amidine-protected cytidine and deoxycytidine. (3) The high-yield synthesis of completely protected nucleosides using amidines makes these reagents ideal for syntheses involving modified bases usually available in limited quantities. This concept was demonstrated with 5-methyldeoxycytidine.

Currently, polymer-supported synthesis of deoxyoligonucleotides involves the condensation of activated deoxymononucleotides with a deoxynucleoside or deoxyoligonucleotide attached covalently to an inorganic, silica-based matrix.[2] This strategy as applied to the triester phosphite method[3] using phosphoramidites[4] as synthons is summarized in Scheme I. Preferentially, deoxynucleoside 3′-phosphoramidites as the diisopropylamino derivatives[4c] **1a–d** are prepared in situ[5] or as isolated synthons,[4] activated with tetrazole, and condensed with a deoxynucleoside or deoxyoligonucleotide such as **2** attached to the support. Condensation yields greater than 99% and the synthesis of deoxyoligonucleotides exceeding 100 bases using only deoxymononucleotide phosphoramidites as synthons have been described.[6]

The phosphoramidite methodology now appears to be limited by the stability of the growing polymer-bound deoxyoligonucleotide toward various reagents used during these lengthy syntheses. In particular, acid-catalyzed depurination[7] of 6-*N*-benzoyldeoxyadenosine[8] (d(bzA)) during acidic removal of the bis(*p*-anisylphenyl)methyl group (DMT, trivially called dimethoxytrityl) can greatly reduce the overall yield.[6] Furthermore, as a result of adenine depurination, a heterogeneous mixture of 5′-dimethoxytrityl deoxyoligonucleotides is present at the conclusion of a synthesis. Thus purification, especially with chromatographic methods based on the presence of the hydrophobic DMT group (principally reverse-phase HPLC[2c,9]), becomes more difficult due to heterogeneity of DMT-containing species. One approach to solving this problem has involved modification of the acidic detritylation conditions. Lewis acids such as $ZnBr_2$,[2c,10] protic acids in mixed solvents,[11] and the use of benzenesulfonic acid, trichloroacetic acid (TCA), and trifluoroacetic acid (TFA) with variable acidity[12] were observed in each case to decrease depu-

Scheme I. General DNA Synthesis Strategy



(1) This is paper 16 in a series on nucleotide chemistry. Paper 15: Caruthers, M. H.; McBride, L. J.; Bracco, L. P.; Dubendorff, J. W. *Nucleosides & Nucleotides* **1985**, *4*, 95. This research was supported by the National Institutes of Health (Grant GM25680) and partially by Upjohn Graduate Fellowships to L.J.M. and J.W.D. The one-letter symbols for nucleotides and the symbols for polynucleotides are according to the IUPAC–IUB Commission on Biochemical Nomenclature Recommendations [*Biochemistry* **1970**, *9*, 4022]. New protecting groups on the base exocyclic amines were abbreviated as follows: aca, *N,N*-dimethylacetamidine; dnb, di-*n*-butylformamidine; pya, *N*-methylpyrrolidine amidine; dmf, *N,N*-dimethylformamidine.

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rination. Also, various 5′-protecting groups were developed which can be removed more selectively than the DMT group. For example, structural modifications of the DMT group increased its lability under acidic[8] or even basic[13] conditions. Recently *p*-phenylazophenyloxycarbonyl was reintroduced as a 5′-protecting group since it can be removed under nonacidic conditions.[14]

Because the DMT group is ideally suited for protecting the nucleoside 5′-hydroxyl and can be removed rapidly by using relatively mild acids, recent efforts have focused on developing new adenine protecting groups which stabilize the glycosidic bond of deoxyadenosine in the presence of acid. Preliminary communications have introduced the phthaloyl,[15] amidine,[16] succinoyl,[17] and dibenzoyl[18] protecting groups for this purpose. This paper summarizes synthesis and depurination studies with amidine-protected deoxyadenosine and adenine-containing deoxyoligonucleotides.

Because of the facile synthetic methods associated with amidine protection of deoxyadenosine, procedures extending the use of amidines to deoxycytidine, cytidine, 5-methyldeoxycytidine, and deoxyguanosine have been developed and will be described. Amidine protection of cytidine was shown to be especially useful since pyrimidine ring substitution with alkylamines, which normally occurs with amide-protected cytosine,[19] did not occur with amidines. Amidine-protected derivatives of deoxyguanosine and deoxycytidine as well as deoxyadenosine were also shown to be compatible as synthons for preparing deoxyoligonucleotides by using the phosphoramidite methodology.

Experimental Section

General Procedures. Nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Varian EM-390 with tetramethylsilane as internal reference. ^{31}P and ^{13}C NMR spectra were recorded on a Brucker WM-250 with 85% (aqueous) phosphoric acid and tetramethylsilane as external references. Downfield chemical shifts were recorded as positive values for ^{31}P NMR. Ultraviolet spectra were recorded on a Cary 17 spectrophotometer. Elemental analyses were performed by Galbraith Laboratories Inc., Knoxville, TN. Enzymes were purchased from Bethesda Research Laboratories. [γ-^{32}P]ATP was purchased from New England Nuclear Research Products.

Thin-layer chromatography (TLC) was performed on EM plastic-backed sheets (silica gel 60 F_{254}, 0.2 mm) in chloroform/methanol/3% acetic acid (aqueous) (3:2:1, the organic phase) (solvent A), hexanes/acetone/triethylamine (40:55:5) (solvent B), or dichloromethane containing 5–20% methanol (solvent C). Preparative chromatography was performed on EM silica gel 60, 230–400 mesh. Evaporations were carried out at 40 °C or lower by using an aspirator or an oil vacuum pump. Solids were dried at 25 °C (0.02 mmHg). Unless specified, reactions were carried out at room temperature.

Pyridine, dichloromethane, *N*-methylpyrrolidone, di-*n*-butylamine, ethylenediamine, and benzene were freshly distilled over CaH_2 (solid). Triethylamine was distilled over toluenesulfonyl chloride followed by CaH_2. Acetonitrile was distilled over P_2O_5 (solid) followed by CaH_2. Hexanes and *n*-pentane were distilled. Diethyl ether was purchased from Mallinckrodt. Ethanol and methanol (spectro grade) were dried over 3-Å molecular sieves regenerated at 200 °C (1 mmHg). Dichloroacetic acid (DCA) was freshly distilled at reduced pressure to remove HCl. TFA was freshly distilled over P_2O_5 (solid). TCA was purchased from Aldrich and used directly.

2′-Deoxythymidine, 2′-deoxyadenosine, and 2′-deoxyguanosine were purchased from Vega Biochemicals. 2′-Deoxycytidine was purchased from Aldrich. 5-Methyl-2′-deoxycytidine (**15c**) was purchased from Sigma Chemicals. Deoxyoligonucleotides were synthesized as described previously.[4,5] 6-*N*-(*N*-Methylpyrrolidin-2-ylidene)-2′-deoxyadenosine (**5d**) and 6-*N*-((dimethylamino)methylene)-2′-deoxyadenosine (**5a**) were prepared according to published procedures.[16,20] Amidine-protected deoxynucleosides **5b**, **11a**, and **11d** were tritylated to **7b**, **12a**, and **12d**, respectively, by using published procedures.[8] Di-*p*-methoxytrityl chloride (DMTrCl) was purchased from Aldrich and recrystallized from hexanes containing acetyl chloride. Tetrazole was purified by sublimation (110 °C, 12 mmHg). Bis(diisopropylamino)methoxyphosphine[5b] and silica gel[2a,4b] or controlled pore glass[2g] (CPG) linked deoxynucleosides were prepared according to published procedures. *p*-(Dimethylamino)pyridine (DMAP) was purchased from Aldrich. Diisopropylammonium tetrazolide[5b] was prepared in near quantitative yield by dissolving tetrazole (4 mmol, 280 mg) in 10 mL of dry acetonitrile. Diisopropylamine (8 mmol, 809 mg) was added with stirring. The product was collected by filtration, washed with dry acetonitrile, and dried in a vacuum oven (40 °C), yielding a white crystalline solid.

Synthesis of 1-*N*-Methyl-2′-deoxyguanosine (14). 2′-Deoxyguanosine dihydrate (**9**) (1.0 mmol, 0.30 g) was refluxed with *N*,*N*-dimethylacetamide dimethyl acetal (10 mmol, 1.5 mL) in 5 mL of methanol for 24 h. The reaction was quenched with 0.5 mL of water, and the reaction mixture was concentrated to ca. 1 mL, transferred into a Teflon-stoppered tube containing concentrated ammonium hydroxide (4 mL), and heated at 60 °C for 16 h. The ammonia was removed in vacuo, methanol was added, the mixture filtered, and the product isolated by crystallization at −20 °C, yielding a white solid (105 mg, 38%). ^{13}C NMR (d_6-Me_2SO) δ 156.3, 154.1, 148.8, 135.2, 115.7, 87.4, 82.3, 70.6, 61.6, 27.9 (N^1–CH_3). ^{1}H NMR (d_6-Me_2SO) δ 8.0 (s, 1, H_8), 7.1 (broad s, 2, $-NH_2$), 6.2 (t, $J_{apparent}$ = 7 Hz, 1, $H_{1'}$), 5.3 (d, 1, 3′-OH), 4.9 (t, 1, 5′-OH), 4.4 (m, 1, $H_{3'}$), 3.8 (m, 1, $H_{4'}$), 3.5 (m, 2, $H_{5',5''}$), 3.3 (s, 3, N–CH_3), 2.7–2.1 (m, 2, $H_{2',2''}$).

Synthesis of Amide Acetals. *N*,*N*-Dimethylformamide dimethyl (**4a**) and diethyl (**10a**) acetals were purchased from Aldrich. *N*,*N*-Di-*n*-butylformamide dimethyl acetal (**4b**) was prepared as described previously.[16b] *N*,*N*-Dimethylacetamide dimethyl acetal (**4c**) was purchased from Aldrich and distilled at reduced pressure. *N*-Methyl-2,2-diethoxypyrrolidine (**10d**) was prepared from published procedures.[21]

***N*-Methyl-2,2-dimethoxypyrrolidine (4d).** *N*-Methylpyrrolidone (0.27 mol, 26 mL) and dimethyl sulfate (0.27 mol, 26 mL) were heated for 1 h at 90 °C. This oil was added dropwise over 1 h into 250 mL of methanol containing dissolved Na (0.37 mol, 8.5 g) with cooling in a −10 °C bath. The mixture was filtered under argon, concentrated at atmospheric pressure, taken up in 250 mL of diethyl ether, filtered again, and the product collected by distillation (7 mmHg, 40–42 °C) to give 16.1 g (41%) of a colorless liquid (d = 0.96). ^{1}H NMR ($CDCl_3$) δ 3.2 (s, 6, O–CH_3), 2.8 (t, J = 6 Hz, 2, N–CH_2), 2.4 (s, 3, N–CH_3), 2.0–1.5 (m, 4, C–CH_2–CH_2–C).

***N*,*N*-Di-*n*-butylformamide Diethyl Acetal (10b).** Di-*n*-butylamine (0.12 mol, 20 mL) and *N*,*N*-dimethylformamide diethyl acetal (0.12 mol, 20 mL) were refluxed under argon from 100 to 160 °C over 48 h. Two fractional distillations (111–113 °C, 12 mmHg) yielded 13.5 g (49%) of a colorless liquid (d = 0.83). ^{1}H NMR ($CDCl_3$) δ 4.6 (s, 1, N=C—H), 3.5 (q, J = 7 Hz, 4, O–CH_2–), 2.6 (t, J = 6 Hz, 4, N(–CH_2–)$_2$), 1.6–0.8 (m, 20, O–C–CH_3, CH_2–CH_2–CH_3).

***N*,*N*-Dimethylacetamide Diethyl Acetal (10c).** To *N*,*N*-dimethylacetamide dimethyl acetal (**4c**) (0.13 mol, 20 mL) was added ethanol (20 mL), and the alcohols were removed by distillation at atmospheric pressure. This addition and removal of alcohol was repeated 5 times. The product was collected by distillation (44–45 °C, 8 mmHg) to give 16.9 g (81%) of a colorless liquid (d = 0.86). ^{1}H NMR ($CDCl_3$) δ 3.4 (q, J = 7 Hz, 4, O–CH_2–), 2.2 (s, 6, N(CH_3)$_2$), 1.2 (s, 3, N–C–CH_3), 1.1 (t, J = 7 Hz, 6, C–CH_3).

Synthesis of Amidine Derivatives. 6-*N*-((Di-*n*-butylamino)-methylene)-2′-deoxyadenosine (5b). 2′-Deoxyadenosine hydrate (**3**) (2.0 mmol, 0.54 g) was coevaporated 3 times with pyridine. Methanol (4 mL) and *N*,*N*-di-*n*-butylformamide dimethyl acetal (**4b**) (3.0 mmol, 0.61 g) were added, and the mixture was stirred for 2 h. The solution was concentrated and flash chromatographed on 25 g of silica gel with 6% methanol in CH_2Cl_2. Concentration of the eluate yielded a white foam (0.68 g, 87%). Mass spectrum, m/e 390 (M^+), 275 (16%), 274 (45%), 273 (13%). ^{1}H NMR ($CDCl_3$) δ 9.0 (s, 1, N=C—H), 8.5 (s, 1, H_8), 8.0 (s, 1, H_2), 6.4 (m, 1, $H_{1'}$), 4.8 (m, 1, $H_{3'}$), 4.2 (m, 1, $H_{4'}$), 3.9–3.3 (m, 6, N(–CH_2–)$_2$ and $H_{5',5''}$), 3.2–2.2 (m, 2, $H_{2',2''}$), 1.8–1.1 (m, 8, N(–C–CH_2–CH_2–)$_2$), 0.9 (m, 6, (–C–CH_3–)$_2$). Anal. Calcd for

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$C_{19}H_{30}N_6O_3$: C, 58.44; H, 7.74; N, 21.52. Found: C, 57.60; H, 7.48; N, 21.55.

6-*N*-(1-(Dimethylamino)ethylene)-2′-deoxyadenosine (5c). 2′-Deoxyadenosine hydrate (**3**) (2.0 mmol, 0.54 g) was coevaporated 3 times with pyridine. *N,N*-Dimethylacetamide dimethyl acetal (**4c**) (5.0 mmol, 0.73 mL) was added, and the mixture was stirred in 2 mL of methanol at 40 °C for 18 h. The solution was concentrated and flash chromatographed on 20 g of silica gel with 8% methanol in CH_2Cl_2. Initial product fractions presumably contained the 6-*N*-methylimino ester[22] (**6**) and were discarded. Fractions containing only **5c** were concentrated to a foam (0.45 g, 70%). Mass spectrum, *m/e* 320 (M^+), 203 (15%), 204 (64%), 205 (15%). ^1H NMR ($CDCl_3$) δ 8.6 (s, 1, H_8), 8.0 (s, 1, H_2), 6.4 (m, 1, $H_{1'}$), 4.8 (m, 1, $H_{3'}$), 4.2 (m, 1, $H_{4'}$), 3.8 (m, 2, $H_{5',5''}$), 3.2 (broad s, 6, $N(CH_3)_2$), 3.1–2.2 (m, 2, $H_{2',2''}$), 2.1 (s, 3, N=C—CH_3). ^{13}C NMR (D_2O, pH 11) δ 164.9, 161.1, 153.2, 150.8, 142.5, 126.5, 88.4, 85.6, 72.1, 62.7, 40.1, 39.2 (broad, integrates as 2), 18.1. Anal. Calcd for $C_{14}H_{20}N_6O_3$: C, 52.49; H, 6.29; N, 26.23. Found: C, 52.29; H, 6.11; N, 24.78.

5′-*O*-(Di-*p*-methoxytrityl)-6-*N*-(1-(dimethylamino)ethylidene)-2′-deoxyadenosine (7c). 2′-Deoxyadenosine hydrate (**3**) (6.0 mmol, 1.6 g) was coevaporated 3 times with pyridine. *N,N*-Dimethylacetamide dimethyl acetal (4c) (18 mmol, 2.7 mL) and methanol (6 mL) were added. After 3 days of stirring, the solution was quenched with H_2O (0.3 mL), concentrated, coevaporated 3 times with pyridine, and taken up in pyridine (50 mL). DMTrCl (7.2 mmol, 2.42 g) was added with stirring. After 90 min the reaction mixture was quenched with methanol (0.05 mL), concentrated to an oil, and dissolved in 50 mL of CH_2Cl_2. The organic phase was extracted with 50 mL of 5% Na_2CO_3 (aqueous) followed by 50 mL of brine. After a 50-mL back-extraction of the aqueous layers, the combined organic fractions were concentrated to a foam and loaded onto a silica gel column (100 g). The product was eluted with CH_2Cl_2/pyridine (99.5:0.5) by using a methanol gradient (0–4%) and medium pressure. The fractions containing only product were concentrated to a foam, precipitated from CH_2Cl_2 (18 mL) into 900 mL of hexanes/diethyl ether (2:1), filtered, and dried to constant weight, yielding a white powder (2.95 g, 79%). ^1H NMR ($CDCl_3$) δ 8.6 (s, 1, H_8), 8.1 (s, 1, H_2), 7.5–6.7 (m, 13, aryl), 6.5 (t, $J_{apparent}$ = 7 Hz, 1, $H_{1'}$), 4.7 (m, 1, $H_{3'}$), 4.1 (m, 1, $H_{4'}$), 3.8 (s, 6, OCH_3), 3.4 (m, 2, $H_{5',5''}$), 3.1 (broad s, 6, $N(CH_3)_2$), 2.9–2.5 (m, 2, $H_{2',2''}$), 2.1 (s, 3, C–CH_3). Anal. Calcd for $C_{35}H_{38}N_6O_5$: C, 67.50; H, 6.15; N, 13.50. Found: C, 67.27; H, 6.34; N, 13.32.

5′-*O*-(Di-*p*-methoxytrityl)-6-*N*-(*N*-methylpyrrolidin-2-ylidene)-2′-deoxyadenosine (7d). 2′-Deoxyadenosine hydrate (**3**) (6.0 mmol, 1.6 g) was coevaporated 3 times with pyridine. *N*-Methyl-2,2-dimethoxypyrrolidine (**4d**) (7.8 mmol, 1.2 mL) and methanol (10 mL) were added. After 2 h, the reaction mixture containing **5d** was quenched with H_2O (0.1 mL), concentrated, coevaporated 3 times with pyridine, and dissolved in 50 mL of pyridine, and DMTrCl (7.2 mmol, 2.43 g) was added with stirring. After 2 h, the reaction mixture was quenched with methanol (0.1 mL), concentrated to an oil, dissolved in 50 mL of CH_2Cl_2, and extracted with 50 mL of 5% Na_2CO_3 (aqueous) followed by 50 mL of brine. After a 50-mL back-extraction of the aqueous phases, the combined organic extracts were concentrated, loaded onto a silica gel column (100 g), and eluted with CH_2Cl_2/pyridine (99.5:0.5) by using a methanol gradient (0–4%) and medium pressure. The fractions containing only product were concentrated to a foam, precipitated from CH_2Cl_2 (15 mL) into 900 mL of *n*-pentane/diethyl ether (2:1), filtered, and dried to constant weight, yielding a white powder (3.4 g, 89%). ^1H NMR ($CDCl_3$) δ 8.5 (s, 1, H_8), 8.0 (s, 1, H_2), 7.5–6.8 (m, 13, aryl), 6.5 (t, $J_{apparent}$ = 7 Hz, 1, $H_{1'}$), 4.7 (m, 1, $H_{3'}$), 4.2 (m, 1, $H_{4'}$), 3.8 (s, 6, OCH_3), 3.6–3.3 (m, 4, $H_{5',5''}$ and N–CH_2), 3.1 (s, 3, N–CH_3), 2.9 (t, $J_{apparent}$ = 8 Hz, 2, N=C—CH_2–), 2.9–2.4 (m, 2, $H_{2',2''}$), 2.0 (m, 2, N–C–CH_2). Anal. Calcd for $C_{36}H_{38}N_6O_5$: C, 68.12; H, 6.04; N, 13.25. Found: C, 67.56; H, 6.32; N, 12.47.

2-*N*-((Dimethylamino)methylene)-2′-deoxyguanosine (11a). 2′-Deoxyguanosine dihydrate (**9**) (8.0 mmol, 2.4 g) and *N,N*-dimethylformamide dimethyl acetal (**4a**) (32 mmol, 4.2 mL) were stirred in methanol (20 mL) for 60 h. Filtration gave a white powder which was washed with methanol and dried to constant weight (2.6 g, 101%). Mass spectrum, *m/e* 206 (48%). ^1H NMR (d_6-Me_2SO, D_2O) δ 8.7 (s, 1, N=C—H), 8.1 (s, 1, H_8), 6.3 (t, $J_{apparent}$ = 7 Hz, 1, $H_{1'}$), 4.4 (m, 1, $H_{3'}$), 3.8 (m, 1, $H_{4'}$), 3.6 (m, 2, $H_{5',5''}$), 3.2–3.0 (2 s, 6, $N(CH_3)_2$), 2.8–2.0 (m, 2, $H_{2',2''}$). Anal. Calcd for $C_{13}H_{18}N_6O_4$: C, 48.44; H, 5.63; N, 26.07. Found: C, 48.24; H, 5.54; N, 26.12.

2-*N*-((Di-*n*-butylamino)methylene)-2′-deoxyguanosine (11b). 2′-Deoxyguanosine dihydrate (**9**) (2.0 mmol, 0.61 g) was coevaporated 3 times with pyridine. Ethanol (4 mL) and *N,N*-di-*n*-butylformamide diethyl acetal (**10b**) (3.0 mmol, 0.70 g) were added, and the mixture was stirred for 16 h. The solution was concentrated, applied to 25 g of silica gel, and eluted by using flash chromatography with 7% methanol in CH_2Cl_2. Product fractions were concentrated to a white foam (0.74 g, 91%). Mass spectrum, *m/e* 291 (89%). ^1H NMR ($CDCl_3$, D_2O) δ 8.6 (s, 1, N=C—H), 8.1 (s, 1, H_8), 6.4 (t, $J_{apparent}$ = 8 Hz, 1, $H_{1'}$), 4.8 (m, 1, $H_{3'}$), 4.2 (m, 1, $H_{4'}$), 3.8 (m, 2, $H_{5',5''}$), 3.3 (m, 4, $N(-CH_2-)_2$), 2.9–2.2 (m, 2, $H_{2',2''}$), 1.8–1.1 (m, 8, $N(-C-CH_2CH_2)_2$), 0.9 (m, 6, CH_3). Anal. Calcd for $C_{19}H_{30}N_6O_4$: C, 56.14; H, 7.44; N, 20.68. Found: C, 55.53; H, 7.28; N, 19.72.

2-*N*-(1-(Dimethylamino)ethylidene)-2′-deoxyguanosine (11c). 2′-Deoxyguanosine dihydrate (**9**) (2.0 mmol, 0.61 g) and *N,N*-dimethylacetamide diethyl acetal (**10c**) (8.0 mmol, 1.3 g) were stirred in dry ethanol (8 mL) for 5 days. The product was filtered, washed with CH_2Cl_2, and dried to constant weight, yielding a white powder (0.62 g, 92%). Mass spectrum, *m/e* 220 (42%), 219 (21%). ^1H NMR (d_6-Me_2SO, D_2O) δ 8.1 (s, 1, H_8), 6.2 (t, $J_{apparent}$ = 7 Hz, 1, $H_{1'}$), 4.4 (m, 1, $H_{3'}$), 3.8 (m, 1, $H_{4'}$), 3.5 (m, 2, $H_{5',5''}$), 3.0 (s, 6, $N(CH_3)_2$), 2.7–2.4 (m, 2, $H_{2',2''}$), 2.2 (s, 3, C–CH_3). Anal. Calcd for $C_{14}H_{20}N_6O_4$: C, 49.99; H, 5.99; N, 24.98. Found: C, 50.16; H, 6.01; N, 24.95.

2-*N*-(*N*-Methylpyrrolidin-2-ylidene)-2′-deoxyguanosine (11d). 2′-Deoxyguanosine dihydrate (**9**) (2.0 mmol, 0.61 g) and *N*-methyl-2,2-diethoxypyrrolidine (**10d**) (8.0 mmol, 1.3 g) were stirred in dry ethanol (8 mL) for 48 h. The product was filtered, washed with ethanol, and dried to constant weight to yield a white powder (0.59 g, 85%). Mass spectrum, *m/e* 232 (63%), 231 (28%). ^1H NMR (d_6-Me_2SO, D_2O) δ 8.1 (s, 1, H_8), 6.3 (t, $J_{apparent}$ = 7 Hz, 1, $H_{1'}$), 4.4 (m, 1, $H_{3'}$), 3.8 (m, 1, $H_{4'}$), 3.7–3.3 (m, 4, $H_{5',5''}$ and N–CH_2–), 3.2–2.8 (m, 2, N=C—CH_2), 3.0 (s, 3, N–CH_3), 2.8–1.8 (m, 4, $H_{2',2''}$ and N–C–CH_2). Anal. Calcd for $C_{15}H_{20}N_6O_4$: C, 51.72; H, 5.79; N, 24.13. Found: C, 51.69; H, 5.89; N, 23.93.

5′-*O*-(Di-*p*-methoxytrityl)-2-*N*-((di-*n*-butylamino)methylene)-2′-deoxyguanosine (12b). 2′-Deoxyguanosine dihydrate (**9**) (6.0 mmol, 1.8 g) was coevaporated 3 times with pyridine. *N,N*-Di-*n*-butylformamide diethyl acetal (**10b**) (9.0 mmol, 2.1 mL) and ethanol (12 mL) were added. After being stirred for 16 h, the reaction mixture was quenched with H_2O (0.1 mL), concentrated, coevaporated 3 times with pyridine, and dissolved in 50 mL of pyridine, and DMTrCl (7.2 mmol, 2.42 g) was added with stirring. After 90 min, the reaction mixture was quenched with methanol (0.05 mL), concentrated, taken up in CH_2Cl_2 (50 mL), and extracted with 50 mL of 5% Na_2CO_3 (aqueous) followed by 50 mL of brine. After a 50-mL back-extraction of the aqueous phases, the combined organic extracts were concentrated to a foam, loaded onto a silica gel column (100 g), and eluted with CH_2Cl_2/pyridine (99.5:0.5) by using a methanol gradient (0–3%) and medium pressure. Product-containing fractions were concentrated to a foam and precipitated from CH_2Cl_2 (18 mL) into 1.0 L of *n*-pentane/diethyl ether (2:1). Filtration and drying to constant weight yielded the product as a white powder (3.3 g, 78%). ^1H NMR ($CDCl_3$) δ 9.0 (s, 1, N–H), 8.7 (s, 1, N=C—H), 7.8 (s, 1, H_8), 7.6–6.7 (m, 13, aryl), 6.5 (t, $J_{apparent}$ = 7 Hz, 1, $H_{1'}$), 4.7 (m, 1, $H_{3'}$), 4.2 (m, 1, $H_{4'}$), 3.8 (s, 6, OCH_3), 3.7–3.2 (m, 6, $H_{5',5''}$ and $N(-CH_2-)_2$), 2.5 (m, 2, $H_{2',2''}$), 1.8–1.1 (m, 8, $N(-C-CH_2CH_2-)_2$), 0.9 (m, 6, $-CH_3$). Anal. Calcd for $C_{40}H_{48}N_6O_6$: C, 67.78; H, 6.83; N, 11.86. Found: C, 67.48; H, 6.97; N, 11.94.

5′-*O*-(Di-*p*-methoxytrityl)-2-*N*-(1-(dimethylamino)ethylidene)-2′-deoxyguanosine (12c). 2-*N*-(1-(Dimethylamino)ethylidene)-2′-deoxyguanosine (**11c**) (6.0 mmol, 2.0 g) was coevaporated 3 times with pyridine. Pyridine (50 mL) and DMTrCl (7.2 mmol, 2.4 g) were added with stirring. After 1 h the reaction mixture was quenched with methanol (0.1 mL), concentrated to an oil, taken up in CH_2Cl_2 (50 mL), and extracted with 50 mL of 2% Na_2CO_3 (aqueous) followed by 50 mL of brine. After two 50-mL back-extractions of the aqueous phases, the combined organic extracts were concentrated to a foam, loaded onto a silica gel (100 g) column, and eluted with CH_2Cl_2/pyridine (99.5:0.5) by using a methanol gradient (0–5%) and medium pressure. The product fractions were concentrated to a foam and precipitated with CH_2Cl_2 (15 mL) into 1.0 L of *n*-pentane/diethyl ether (1:1). Filtration and drying to constant weight yielded the product as a white powder (3.1 g, 81%). ^1H NMR ($CDCl_3$) δ 9.7 (s, 1, N–H), 7.8 (s, 1, H_8), 7.6–6.8 (m, 13, aryl), 6.4 (t, $J_{apparent}$ = 7 Hz, 1, $H_{1'}$), 4.7 (m, 1, $H_{3'}$), 4.2 (m, 1, $H_{4'}$), 3.7 (s, 6, OCH_3), 3.4 (m, 2, $H_{5',5''}$), 3.0 (s, 6, $N(CH_3)_2$), 2.5 (m, 2, $H_{2',2''}$), 2.2 (s, 3, C–CH_3). Anal. Calcd for $C_{35}H_{38}N_6O_6$: C, 65.81; H, 6.00; N, 13.16. Found: C, 64.95; H, 6.14; N, 12.72.

4-*N*-((Di-*n*-butylamino)methylene)-2′-deoxycytidine (16b). 2′-Deoxycytidine hydrate (**15a**) (1.0 mmol, 0.25 g) was coevaporated 3

(22) A small amount of this impurity was purified and found to have a mass spectrum, *m/e* 307 (M^+). This side product was conveniently converted to its 5′-DMT derivative during a 30-mmol scale preparation of **7c**. The DMT-containing side product was isolated by silica gel column chromatography (<1% yield). ^1H NMR ($CDCl_3$) δ 8.7 (s, 1, H_8), 8.2 (s, 1, H_2), 7.5–6.7 (m, 13, aryl), 6.5 (t, $J_{apparent}$ = 7 Hz, 1, $H_{1'}$), 4.7 (m, 1, $H_{3'}$), 4.2 (m, 1, $H_{4'}$), 3.9 (s, 3, N=C—OCH_3), 3.8 (s, 6, OCH_3), 3.4 (m, 2, $H_{5',5''}$), 2.9–2.5 (m, 2, $H_{2',2''}$), 2.0 (s, 3, N=C—CH_3).

times with pyridine. Methanol (2 mL) and *N,N*-di-*n*-butylformamide dimethyl acetal (**4b**) (1.3 mmol, 0.27 g) were added, and the mixture was stirred for 2 h. The reaction mixture was concentrated, taken up in 25 mL of CH_2Cl_2, extracted twice with 5% $NaHCO_3$ (aqueous), dried over anhydrous Na_2SO_4 (solid), concentrated to 3 mL, and precipitated into 125 mL of hexanes to yield a white powder (0.33 g, 90%). Mass spectrum, *m/e* 366 (M^+), 251 (15%), 250 (24%). ^1H NMR ($CDCl_3$) δ 8.8 (s, 1, N=C—H), 8.1 (d, $J_{5,6}$ = 8 Hz, H_6), 6.2 (t, $J_{apparent}$ = 7 Hz, 1, $H_{1'}$), 6.1 (d, $J_{5,6}$ = 8 Hz, 1, H_5), 4.6 (m, 1, $H_{3'}$), 4.0 (m, 1, $H_{4'}$), 3.9 (m, 2, $H_{5',5''}$), 3.7–3.2 (m, 4, $N(-CH_2-)_2$), 2.4 (m, 2, $H_{2',2''}$), 1.8–1.1 (m, 8, $N(-C-CH_2CH_2-)_2$), 0.9 (m, 6, $-CH_3$). Anal. Calcd for $C_{18}H_{30}N_4O_4$: C, 58.99; H, 8.25; N, 15.29. Found: C, 59.01; H, 8.22; N, 15.21.

4-*N*-(*N*-Methylpyrrolidin-2-ylidene)-2′-deoxycytidine (16d). 2′-Deoxycytidine hydrate (**15a**) (1.0 mmol, 0.25 g) was coevaporated 3 times with pyridine. *N*-Methyl-2,2-dimethoxypyrrolidine (**4d**) (1.3 mmol, 1.9 g) was added and the mixture stirred in methanol (2 mL) for 1 h. The reaction mixture was concentrated and fractionated by flash chromatography on silica gel with 8% methanol in CH_2Cl_2. Concentration of the product fractions gave a white foam (0.29 g, 94%). Mass spectrum, *m/e* 308 (M^+), 193 (34%), 192 (96%), 191 (75%). ^1H NMR ($CDCl_3$) δ 7.9 (d, $J_{5,6}$ = 8 Hz, 1, H_6), 6.1 (t, $J_{apparent}$ = 7 Hz, 1, $H_{1'}$), 6.0 (d, $J_{5,6}$ = 8 Hz, 1, H_5), 4.6 (m, 1, $H_{3'}$), 4.0 (m, 1, $H_{4'}$), 3.8 (m, 2, $H_{5',5''}$), 3.5 (m, 2, $N-CH_2-$), 3.2 (m, 2, $N=C-CH_2$), 3.0 (s, 3, $N-CH_3$), 2.4 (m, 2, $H_{2',2''}$), 2.0 (m, 2, $N-C-CH_2-$). Anal. Calcd for $C_{14}H_{20}N_4O_4$: C, 54.53; H, 6.54; N, 18.17. Found: C, 54.18; H, 6.34; N, 17.97.

5′-*O*-(Di-*p*-methoxytrityl)-4-*N*-(*N*-methylpyrrolidin-2-ylidene)-2′-deoxycytidine (17a). 2′-Deoxycytidine hydrate (**15a**) (6.0 mmol, 1.47 g) was coevaporated 3 times with pyridine. *N*-Methyl-2,2-dimethoxypyrrolidine (**4d**) (7.8 mmol, 1.1 g) and methanol (10 mL) were added. After 90 min of stirring, the solution was quenched with H_2O (0.05 mL), concentrated, coevaporated 3 times with pyridine, and dissolved in 50 mL of pyridine. DMTrCl (7.2 mmol, 2.43 g) was then added with stirring. After 90 min the solution was quenched with methanol (0.05 mL) and concentrated, and the oil was dissolved in CH_2Cl_2 (50 mL) and extracted with 50 mL of 5% Na_2CO_3 (aqueous) followed by 50 mL of brine. After a 50-mL back-extraction of the aqueous phases, the combined organic extracts were concentrated, loaded onto a silica gel column (100 g), and eluted with CH_2Cl_2/pyridine (99.5:0.5) by using a methanol gradient (0–4%) and medium pressure. The product fractions were concentrated to a foam and precipitated with CH_2Cl_2 (15 mL) into hexanes/diethyl ether (2:1). Filtration and drying to constant weight yielded the product as a white powder (2.92 g, 80%). ^1H NMR ($CDCl_3$) δ 7.9 (d, $J_{5,6}$ = 8 Hz, 1, H_6), 7.5–6.8 (m, 13, aryl), 6.4 (t, $J_{apparent}$ = 7 Hz, 1, $H_{1'}$), 5.8 (d, $J_{5,6}$ = 8 Hz, 1, H_5), 4.5 (m, 1, $H_{3'}$), 4.1 (m, 1, $H_{4'}$), 3.8 (s, 6, OCH_3), 3.6–3.4 (m, 4, $H_{5',5''}$ and $N-CH_2$), 3.2 (t, $J_{apparent}$ = 7 Hz, 2, $N=C-CH_2$), 3.0 (s, 3, $N-CH_3$) 2.7–2.2 (m, 2, $H_{2',2''}$), 2.1 (m, 2, $N-C-CH_2$). Anal. Calcd for $C_{35}H_{38}N_4O_6$: C, 68.83; H, 6.27; N, 9.17. Found: C, 68.60; H, 6.26; N, 9.15.

5′-*O*-(Di-*p*-methoxytrityl)-4-*N*-(*N*-methylpyrrolidin-2-ylidene)-5-methyl-2′-deoxycytidine (17c). 5-Methyl-2′-deoxycytidine (**15c**) (1.9 mmol, 0.45 g) was coevaporated twice with pyridine. *N*-Methyl-2,2-dimethoxypyrrolidine (**4d**) (3.4 mmol, 0.49 g) and absolute ethanol (2 mL) were added. After being stirred for 3 h, the mixture was quenched with water (0.1 mL), concentrated, coevaporated 3 times with pyridine, and dissolved in 10 mL of pyridine. DMTrCl (2.3 mmol, 0.78 g) was then added with stirring. After 90 min, the reaction mixture was quenched with methanol (0.05 mL), concentrated to an oil, redissolved in CH_2Cl_2 (25 mL), and extracted first with 15 mL of 3% Na_2CO_3 (aqueous) and then with 15 mL of brine. After two 15-mL back-extractions of the aqueous phases, the combined organic extracts were concentrated to a foam, loaded onto a 15-g silica gel column, and eluted with CH_2Cl_2/pyridine (99.5:0.5) by using a methanol gradient (0–4%) and medium pressure. The product-containing fractions were concentrated to a foam and precipitated from CH_2Cl_2 into 100 mL of hexanes/diethyl ether (2:1). The white precipitate was collected by filtration and dried to constant weight (0.94 g, 82%). ^1H NMR ($CDCl_3$) δ 7.8 (s, 1, H_6), 7.5–6.8 (m, 13, aryl), 6.5 (t, $J_{apparent}$ = 7 Hz, 1, $H_{1'}$), 4.5 (m, 1, $H_{3'}$), 4.1 (m, 1, $H_{4'}$), 3.8 (s, 6, OCH_3), 3.6–3.4 (m, 4, $H_{5',5''}$ and $N-CH_2$), 3.2–3.0 (m, 5, $N=C-CH_2$ and $N-CH_3$), 2.8–1.9 (m, 4, $H_{2',2''}$ and $N-C-CH_2$), 1.6 (s, 3, C^5-CH_3).

Synthesis of Phosphoramidites. 3′-*O*-((Diisopropylamino)methoxyphosphino)-5′-*O*-(di-*p*-methoxytrityl)-6-*N*-(1-(dimethylamino)ethylidene)-2′-deoxyadenosine (8c). 5′-*O*-(Di-*p*-methoxytrityl)-6-*N*-(1-(dimethylamino)ethylidene)-2′-deoxyadenosine (**7c**) (1.0 mmol, 0.62 g) and diisopropylammonium tetrazolide (0.5 mmol, 84 mg) were dissolved in CH_2Cl_2 (5 mL). Bis(diisopropylamino)methoxyphosphine (1.1 mmol, 0.32 mL) was added with stirring under argon. After 1 h, the reaction mixture was diluted to 25 mL with CH_2Cl_2 and extracted twice with 2% Na_2CO_3 (aqueous) (25 mL) followed by 25 mL of brine. After a 25-mL back-extraction of the aqueous phases, the combined organic fractions were dried over Na_2SO_4 (solid) and concentrated to 5 mL, and the product was precipitated from 200 mL of cold hexanes (−50 °C) to yield, after drying to constant weight, a white powder (0.71 g, 89%). ^{31}P NMR (CH_3CN) δ 148.9, 149.1.

3′-*O*-((Diisopropylamino)methoxyphosphino)-5′-*O*-(di-*p*-methoxytrityl)-2-*N*-(1-(dimethylamino)ethylidene)-2′-deoxyguanosine (13c). 5′-*O*-(Di-*p*-methoxytrityl)-2-*N*-(1-(dimethylamino)ethylidene)-2′-deoxyguanosine (**12c**) (1.0 mmol, 0.64 g) and diisopropylammonium tetrazolide (0.5 mmol, 84 mg) were dissolved in CH_3CN (10 mL), and bis(diisopropylamino)methoxyphosphine (1.1 mmol, 0.32 mL) was added under argon with stirring. After 2 h the reaction mixture was quenched with H_2O (0.06 mmol, 10 μL of a 10% solution in CH_3CN), taken up in CH_2Cl_2 (30 mL), and extracted twice with 20 mL of 2% Na_2CO_3 (aqueous) followed by 20 mL of brine. After a 10-mL back-extraction of the aqueous phases, the combined organic extracts were dried over Na_2SO_4 (solid), concentrated to 6 mL, and precipitated from 250 mL of cold hexanes (−50 °C). The white powder was dried to constant weight (0.78 g, 96%). ^{31}P NMR (CH_3CN) δ 148.7, 148.9.

3′-*O*-((Diisopropylamino)methoxyphosphino)-5′-*O*-(di-*p*-methoxytrityl)-4-*N*-(*N*-methylpyrrolidin-2-ylidene)-2′-deoxycytidine (18). This was prepared analogously to **8c**. ^{31}P NMR (CH_3CN) δ 148.8, 149.1. The two diastereomers in the crude precipitate were separated on 3 g of silica gel (Merck silica gel 60 prep TLC silica) by using 200 mL of ethyl acetate/hexanes/triethylamine (60:40:5) followed by 200 mL (67:33:5) of the same solvent mixture with medium pressure. The initial product-containing fractions were concentrated and lyophilized with benzene to a white foam (compound a) (10 mg, 20% recovered) as were the trailing fractions (compound b) (15 mg, 30% recovered). ^{31}P NMR (CH_3CN) compound a, δ 148.9; compound b, 148.6. Each diastereomer contained less than 8% of the other by ^{31}P NMR spectroscopy. Compound b also was contaminated with 20% hydrolysis products at δ 8.6, while compound a contained approximately 8% hydrolysis products by ^{31}P NMR spectroscopy.

In Situ Synthesis of Phosphoramidites. Deoxynucleoside phosphoramidites were also prepared in situ as 0.1 M solutions in acetonitrile. The following general procedure as outlined for the synthesis of **8c** was used. Bis(diisopropylamino)methoxyphosphine (0.88 mmol, 260 μL) was added to a vacuum-dried mixture of **7c** (0.8 mmol, 0.50 g) and diisopropylammonium tetrazolide (0.44 mmol, 75 mg) in 8.0 mL of dry acetonitrile. After 90 min, TLC on silica using solvent B showed that the reaction was complete. The cloudy mixture was quenched by adding water (0.06 mmol, 10 μL of a 10% aqueous solution in acetonitrile). Deoxynucleoside phosphoramidites (0.1 M) prepared in situ by this method were then used in the synthesis protocol described in Table I.

Chemical Synthesis of Deoxyoligonucleotides. Syntheses of d(G-G-G-A-A-T-T-C-C-C) were performed manually in 2-mL sintered glass funnels[4b,23] by using in situ prepared phosphoramidites **1d**, **8c**, **13c**, and **18** or **1a–d**. The synthesis procedure as published previously[5b] is sum-

Table I. Protocol for the Manual, in Situ Phosphoramidite Synthesis of Deoxyoligonucleotides on Silica Gel or Controlled-Pore Glass

step[a]	reagent or solvent mixture[b]	time
1, 2, 3	CH_2Cl_2	10 s each step
4	DCA/CH_2Cl_2 (1:50, v/v)	3 min
5, 6	CH_2Cl_2	10 s each step
7, 8	CH_3CN (HPLC grade)	10 s each step
9, 10, 11	CH_3CN (dry)[c]	10 s each step
12	0.1 M phosphoramidite prepared in situ (0.2 mL)[c] followed by 0.4 M tetrazole (0.2 mL)[c]	5 min
13	DMAP/THF/lutidine (6:90:10, w/v/v), then 0.1 mL of acetic anhydride	2 min
14	THF/lutidine/H_2O (2:2:1, v/v/v)	1 min
15	0.1 M I_2 in THF/lutidine/H_2O (2:2:1, v/v/v)	30 s
16, 17, 18, 19	CH_3CN	10 s each step

[a] Multiple washes with the same solvent involve filtration between each wash step where sintered glass funnels are used as reaction flasks. [b] Each step volume was 1 mL unless indicated. Usually silica or controlled-pore glass containing a deoxynucleoside (30–40 mmol/g, 25 mg) was used per synthesis. [c] Reagents were in CH_3CN and stored under argon.

(23) Caruthers, M. H.; Beaucage, S. L.; Becker, C.; Efcavitch, J. W.; Fisher, E. F.; Galluppi, G.; Goldman, R. A.; deHaseth, P. L.; Martin, F.; Matteucci, M. D.; Stabinsky, Y. "Genetic Engineering, Vol. 4"; Plenum Press: New York, 1982; p 1–17.

marized in Table I. d(T-T-T-T-T-T-T-T-T-T-bzA-T-T-T-T-T-T-T-T-T-T) and d(T-T-T-T-T-T-T-T-T-T-acaA-T-T-T-T-T-T-T-T-T-T) were synthesized with an Applied Biosystems 380A DNA Synthesizer on controlled-pore glass purchased from Applied Biosystems by a procedure similar to our published methods[4] (see Applied Biosystems users manual as revised August 2, 1983).

Synthetic DNA was freed of protecting groups by using standard conditions:[23] (1) demethylation of the triesters with *p*-dioxane/thiophenol/triethylamine (2:1:1) for 90 min, (2) cleavage from the support with concentrated ammonium hydroxide at room temperature for 4 h, (3) amidine deprotection with concentrated ammonium hydroxide at 62 °C (16 h), and (4) detritylation of the crude product with aqueous 80% acetic acid for 1 h. After enzymatic phosphorylation with T4 kinase and [γ-^{32}P]ATP, the crude product was purified by polyacrylamide gel electrophoresis.[23] A similar procedure was used to deprotect and characterize DNA synthesized from amide-protected deoxynucleoside phosphoramidites (**1a–d**).

Exocyclic Amino Group Deprotection Studies. Deoxynucleoside exocyclic amino protecting groups were removed with either ethylenediamine/phenol or concentrated ammonium hydroxide. Deprotections with ethylenediamine were performed by suspending finely divided, protected deoxynucleosides (approximately 10 mg) in 0.4 mL of freshly prepared ethylenediamine/phenol/water (2:8:1, v/w/v) with heating at 40 °C and frequent vortexing until the solution was homogeneous.[16a] Alternatively, the protected deoxynucleoside (20 mg) was suspended in cold, concentrated ammonium hydroxide (3 mL, 28%) and placed in a cooled, 15-mL screw cap test tube fitted with a Pierce Teflon disk, and the sealed tube was heated at 50 °C until deprotection was complete. Deprotection with concentrated ammonium hydroxide (28%) containing ammonium acetate (10:1, v/w) at 50 °C was performed similarly.[16b] Half-lives for deprotection were estimated by thin-layer chromatography on silica gel in solvents A and C, and the results were confirmed in most cases by ultraviolet spectrophotometry.

Depurination Studies. Depurination kinetics of synthetic deoxyoligothymidine containing an internal, exocyclic amino-protected deoxyadenosine were performed as follows: Deoxyoligonucleotide samples covalently joined to controlled-pore glass (approximately 0.5 mg) were suspended for various times in Microeppendorf tubes containing a solution of TCA/CH_2Cl_2 (3:97, w/v). Samples were then centrifuged, and the acid was decanted. After being washed with dichloromethane, methanol, and ethyl ether, the samples were fully deprotected by using conditions outlined in the section on chemical synthesis of deoxyoligonucleotides, labeled at the 5′-end by using [γ-^{32}P]ATP and T4 kinase, and analyzed by electrophoresis on polyacrylamide gels. The exact deprotection protocol used is described in the section on chemical synthesis of deoxyoligonucleotides. The relative amounts of cleavage product resulting from depurination followed by β-elimination at the apurinic site were estimated by autoradiography and quantitated by cutting the appropriate [^{32}P]-labeled bands from the gel. Radioactivity was monitored in a Beckman Scintillation Counter. The results are reported in Figure 6.

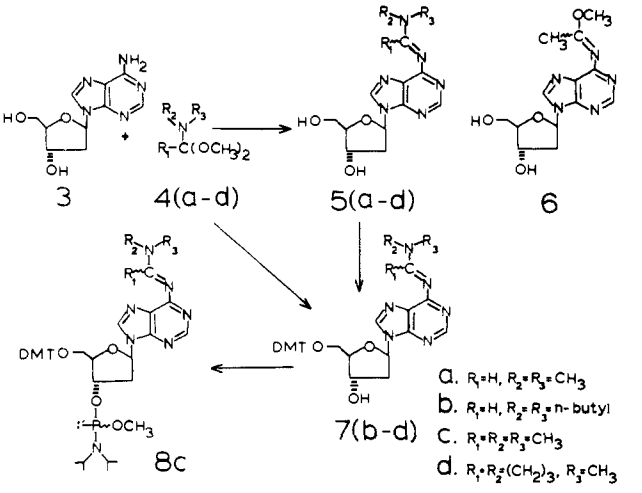
Depurination half-lives of deoxynucleosides attached covalently to Fractosil-500 or controlled-pore glass were also determined. Support-bound samples of various protected deoxynucleosides (5–10 mg containing 30–40 μmol of deoxynucleoside/g) were placed in 3-mL, Teflon-stoppered cuvettes containing DCA/CH_2Cl_2 (1:50, v/v) or TFA/CH_2Cl_2 (1:50, v/v). The ultraviolet absorbance at the λ_{max} for the protected purine as liberated in solution from the support was monitored as a function of time.

Results and Discussion

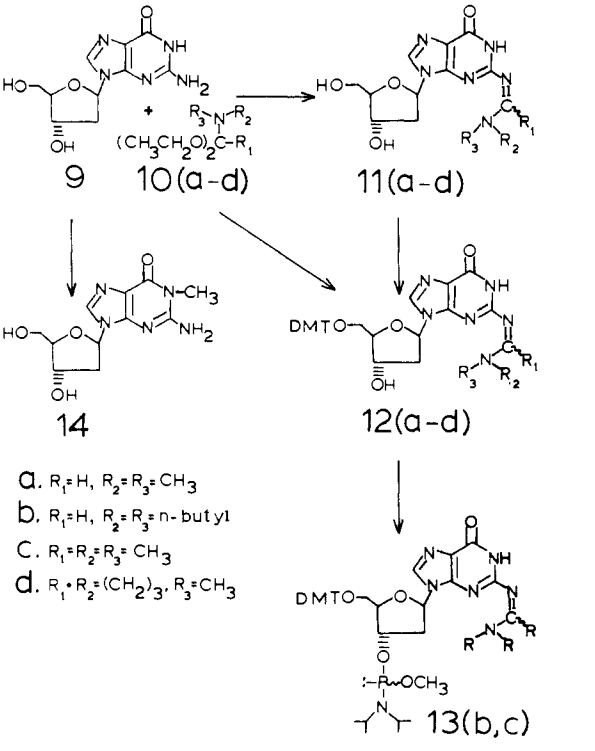
Synthesis of Amidine-Protected Deoxynucleosides. *N,N*-Dimethylformamidine[20] was initially investigated as a depurination-resistant protecting group for deoxyadenosine. As reported previously, the resulting amidine (**5a**) was unstable to various acidic[16a] and basic[16b] conditions used during DNA synthesis. Furthermore, the instability of **5a** on silica gel precluded its use where purification via silica gel chromatography was necessary. These results confirmed our expectations that *N,N*-dimethylformamidine as a protecting group on deoxyadenosine was not compatible with the synthesis pathway summarized in Scheme I. Because of the lability of **5a**, a series of sterically hindered amidines were investigated as protecting groups for **3**.

The synthesis of amidines begins with the amide acetals. Unless commercially available (**4a**, **4c**, **10a**), the amide acetals were prepared via methylation of the appropriate amide with dimethyl sulfate followed by treatment of the salt with the desired sodium alcoholate (**4d**,**10d**),[24] transamidation[16b] of *N,N*-dimethylformamide dimethyl acetal and *N,N*-dimethylformamide diethyl acetal with *N,N*-di-*n*-butylamine (**4b**,**10b**), or repeated distillation of commercially available *N,N*-dimethylacetamide dimethyl acetal with ethanol to form the diethyl acetal (**10c**).

Scheme II. Synthesis of 2′-Deoxyadenosine Amidines



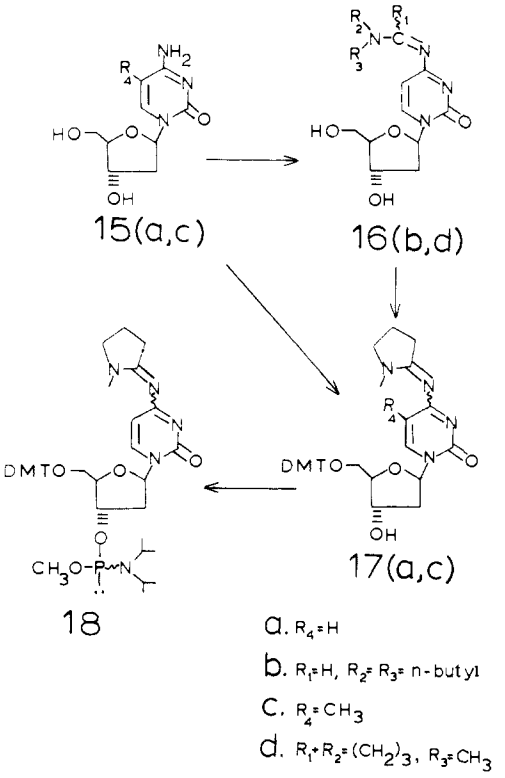
Scheme III. Synthesis of 2′-Deoxyguanosine Amidines



Fully protected amidine deoxynucleosides were synthesized from **3**, **9**, and **15a**,**c** (Schemes II, III, and IV) via one-flask procedures. The dry deoxynucleoside and appropriate amide acetal (**4a–d**, **10a–d**; 1.3–4 equiv) were stirred in a minimum amount of dry methanol or ethanol for 1 h to 5 days. After completion of reactions, water was added to neutralize excess amide acetal, solutions were repeatedly concentrated with dry pyridine to remove water and alcohols, and intermediates **5c**,**d**, **11b**, and **16d** were directly reacted without isolation with dimethoxytrityl chloride in anhydrous pyridine to form the fully protected 5′-dimethoxytrityl-containing compounds (**7b–d**, **12b**, **17a**). After medium-pressure chromatography on silica gel, isolated yields of **7b–d**, **12b**, and **17a** were 78–89%. Unfortunately the *N*-methylpyrrolidine amidine (pya) of deoxycytidine (**17a**) was the only

(24) Bredereck, H.; Simchen, G.; Rebsdat, S.; Kantlekner, W.; Horn, P.; Wahl, R.; Hoffman, H.; Grieshaber, P. *Chem. Ber.* **1968**, *101*, 41.

Scheme IV. Synthesis of 2′-Deoxycytidine and 2′-Deoxy-5-methylcytidine Amidines



amidine derivative of deoxycytidine tested which was stable enough for chromatography on silica gel. Other derivatives of deoxycytidine such as *N,N*-dimethylformamidine, *N,N*-dimethylacetamidine (aca), and *N,N*-di-*n*-butylformamidine (dnb) decomposed slowly during silica gel chromatography. However, since the synthesis of amidine-protected deoxynucleosides was essentially quantitative, derivatives sensitive to silica gel can usually be isolated in high purity simply by precipitation as was done with **16b**. Therefore fully protected amidine derivatives of **3**, **9**, and **15a** can be prepared via one-flask procedures without isolation of intermediates or transient protection of deoxyribose hydroxyl groups as is necessary for amide protecting groups.[17,25]

This highly reliable, one-flask synthesis procedure for protecting deoxynucleosides has also been used for expensive deoxynucleoside analogues such as 5-methyl-2′-deoxycytidine (**15c**), where simplicity and high yields are particularly important considerations. As outlined in the Experimental Section, the conversion of **15c** to the completely protected **17c** gave an overall yield of 82%. When this protection procedure is coupled with the in situ methodology for synthesizing deoxynucleoside phosphoramidites,[5] a very attractive route requiring minimal amounts of starting material is available for preparing synthons of deoxynucleoside analogues for biochemical investigations.[26]

The general susceptibility of the guanine moiety toward electrophilic attack proved initially to be troublesome. For example (Scheme III), treatment of **9** with dimethyl acetals of **4b**, **4c**, and **4d** led to methylation of guanine at 1-N as has been reported previously for alkylation of both purines and pyrimidines at the imide nitrogen by amide acetals.[27] Deprotection with concentrated ammonium hydroxide followed by crystallization of the product from methanol yielded white crystals with ^1H and ^{13}C NMR spectra consistent with 1-*N*-methyl-2′-deoxyguanosine (**14**).[28] However by using amide diethyl acetals (**10b–d**), alkylation of guanine was eliminated as a serious problem. Methylation of **9** by *N,N*-dimethylformamide dimethyl acetal (**4a**) in methanol was not observed, presumably because of the low solubility of the amidine product (**11a**) in the methanolic reaction mixture.

The existence of geometrical isomerization at the amidine double bond was in all cases undetected by thin-layer chromatography. The ^{13}C NMR spectrum of **7d** was also consistent with the predominant presence of one geometric isomer or the rapid interconversion between these isomers.

Phosphoramidites of Amidine Nucleosides. Conversion of amidine-protected deoxynucleosides to phosphoramidites was completed as described previously from either chlorodiisopropylaminomethoxyphosphine[4c,16a] or bis(diisopropylamino)methoxyphosphine.[5b] The preferred procedure involves using bis(diisopropylamino)methoxyphosphine which, unlike the chlorophosphine, is very stable even when water and oxygen are present but can be easily activated by weak acids such as diisopropylammonium tetrazolide.[5b] By use of bis(diisopropylamino)methoxyphosphine, the deoxynucleoside phosphoramidite products are not activated further by the tetrazolide salt and therefore can be isolated without contamination from deoxynucleosidephosphonic acid or 3′,3′-dinucleoside phosphite side products.[4a,5b] After 1–2-h reactions, **8c**, **13c**, and **18** were isolated as essentially homogeneous products by using a standard aqueous extraction and precipitation procedure[4,5] developed previously for amide-protected deoxynucleoside phosphoramidites (**1a–d**). Characterization by ^{31}P NMR spectroscopy of these phosphoramidites showed the expected 1:1 ratio of diastereomers which accounted for at least 95% of the total phosphorus (data not shown). The only major phosphorus contaminating side product as a result of this simple workup was phosphoamidous acid. This compound, produced by reaction of the activated bis(phosphine) with trace amounts of water in the reaction mixture or during the water quench, was not deleterious to the chemistry, and its formation can be used as a method for removing trace water contamination from solvents during the in situ approach to DNA synthesis.[5]

The 1:1 diastereomeric mixture of **18** was observed to be resolvable by TLC on silica gel in solvent system B or by silica gel column chromatography. These results suggest that the deoxynucleoside phosphoramidites do not undergo inversion at room temperature. Furthermore this procedure for isolating diastereomeric deoxynucleoside phosphoramidites should prove to be useful for mechanistic studies of phosphoramidite activation and condensation reactions and for further research involving synthesis and biochemical studies on DNA-containing phosphate analogues having predetermined configurations such as the methyl phosphonates.[29]

Amidine-protected deoxynucleosides can also be used to prepare deoxynucleoside phosphoramidites in situ.[5b] The procedure involves adding bis(diisopropylamino)methoxyphosphine (1.1 equiv) and diisopropylammonium tetrazolide (0.5 equiv) to a dry acetonitrile solution of amidine-protected deoxynucleoside (**7c**, **12c**, or **17a**). The reactions as monitored by TLC were greater than 98% complete in 1–2 h. After addition of water to neutralize excess bis(diisopropylamino)methoxyphosphine, further activation with tetrazole showed, by ^{31}P NMR spectroscopy, less than 2% of the 3′,3′ triphosphite dimer at 139 ppm[4] (Figure 1). The major peak corresponding to activated deoxynucleoside phosphoramidite (presumably the tetrazolide[4c]) was at 126 ppm. Other peaks correspond to phosphoamidous acid at 14 ppm and deoxynucleoside-3′-phosphonic acid at 8 ppm, a product of the phosphoramidite reacting with tetrazole and excess water present from the quench. The lack of any 3′,3′-dinucleoside phosphite dimer confirmed the results from TLC that phosphitylation of amidine-protected deoxynucleoside proceeds to greater than 98% yield. If unreacted deoxynucleoside were present, considerable 3′,3′ dimer would have been expected.

DNA Synthesis. Amidine-protected deoxynucleosides were observed to be stable to reaction conditions (Table I) present

(25) Ti, G. S.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1982**, *104*, 1316.

(26) **17c** has been used to synthesize deoxyoligonucleotides useful for studying the −35 region of *E. coli* promoters (Dubendorff, J.; Caruthers, M. H., unpublished results).

(27) Zemlicka, J., *Collect. Czech. Chem. Commun.* **1970**, *35*, 3572.

(28) Ching-jer Chang; Chi-Gen Zee *Biochemistry* **1981**, *20*, 2657.

(29) Noble, S. A.; Fisher, E. F.; Caruthers, M. H.; *Nucleic Acids Res.* **1984**, *12*, 3387.

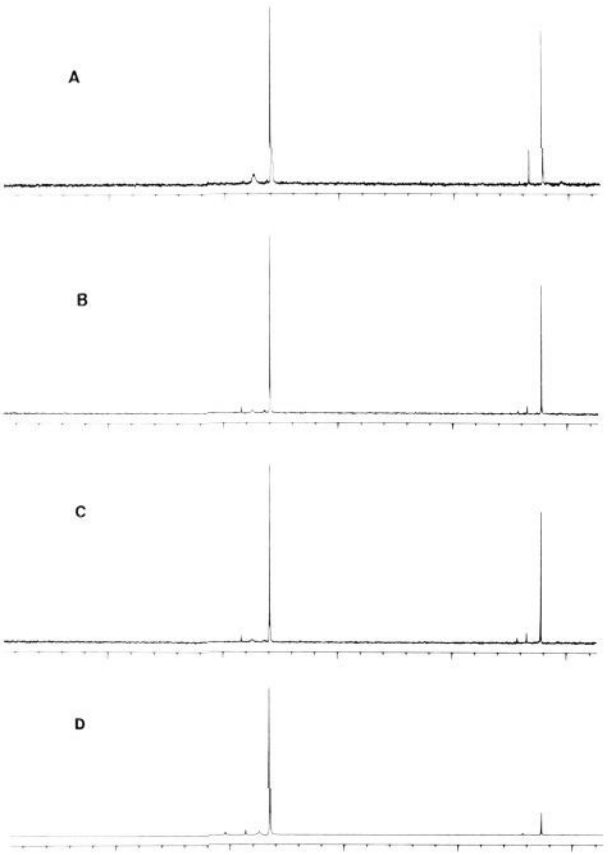


Figure 1. ^{31}P NMR spectra of amidine-protected phosphoramidites activated with tetrazole. Deoxynucleoside phosphoramidites were prepared in situ as described in the Experimental Section (including the aqueous quench). Appropriate phosphoramidites (panel A, **13b**; panel B, **18**; panel C, **8c**; 0.1 mmol in 2.5 mL of CH_3CN) were added to tetrazole (1.1 mmol, 78 mg) and the ^{31}P NMR spectra recorded. Panel D, a control ^{31}P NMR spectrum of dimethoxy(diisopropylamino)phosphine activated with tetrazole was prepared as follows. Bis(diisopropylamino)methoxyphosphine (0.40 mmol, 0.12 mL) was added to 2 mL of CH_3CN containing tetrazole (0.35 mmol, 24 mg). Methanol (0.40 mmol, 16 μL) was then added. After 1 h, more tetrazole (1.2 mmol, 84 mg) was added and the spectrum shown in panel D was taken.

during DNA synthesis. Samples of **7c**, **12c**, and **17a** (amidines which are currently being used for DNA synthesis) or their 3′-benzoyl derivatives were stored in the 0.1 M I_2 oxidation solution, the detritylation conditions, and a solution of tetrazole-activated deoxynucleoside phosphoramidite, **13c**. On the basis of TLC analysis after 24 h, no instability due to oxidation or acid hydrolysis of these amidines to unprotected deoxynucleosides was observed. After 24 h of exposure to the activated deoxynucleoside phosphoramidites, **7c**, **12c**, and **17a** were completely stable as judged by aqueous hydrolysis followed by TLC of the samples. These results suggest that amidines are quite stable to DNA synthesis reaction conditions and can be used for at least 200 successive cycles per each total synthesis.

In order to test the compatibility of amidine-protected deoxynucleoside phosphoramidites as DNA synthons, d(G-G-G-A-A-T-T-C-C-C) was prepared by using both amidine- and amide-protected deoxynucleosides and the manual, in situ method. These two syntheses were performed in sintered glass funnels with 1 μmol of silica-bound deoxynucleoside and the procedure outlined in Table I. Following the detritylation step, the appropriate in situ prepared phosphoramidite (**1d**, **8c**, **13c**, **18**) was added to the silica gel under an argon atmosphere. The steps shown in Table I were repeated until completion of the synthesis. The same procedure was used for synthesizing d(G-G-G-A-A-T-T-C-C) from **1a–d**. Following removal of protecting groups by using the conditions outlined in the Experimental Section and phosphorylation of the 5′-hydroxyl group with [γ-^{32}P]ATP and T4 kinase, the two crude

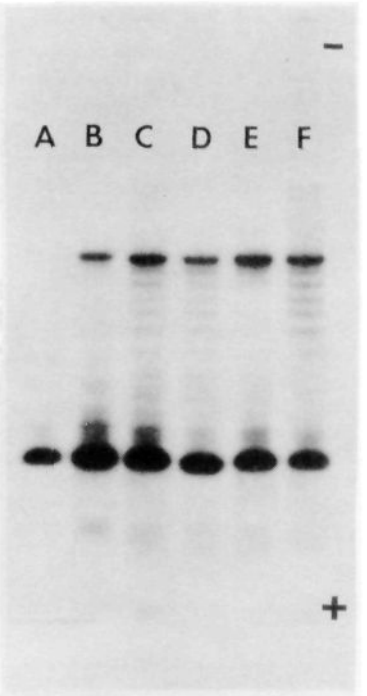


Figure 2. Autoradiogram of a 20% polyacrylamide gel. 5′-d(G-G-G-A-A-T-T-C-C-C) was prepared with different protocols by the in situ method. After completion of each synthesis, protecting groups were removed. The total hydrolysates from supports were radioactively labeled by using [γ-^{32}P]ATP and T4 kinase, loaded into various lanes, and analyzed by electrophoresis. Lanes C and F are syntheses on Fractosil-500 and controlled-pore glass, respectively, using **1d**, **8c**, **13c**, and **18**. Lanes B and E are the respective reverse-phase HPLC purified products. Lane D is a synthesis with **1a–d** on Fractosil-500. Lane A is [γ-^{32}P]ATP.

reaction mixtures were analyzed by polyacrylamide gel electrophoresis (Figure 2). Essentially identical results were obtained in each case (35–40% isolated yield of product by reverse-phase HPLC), suggesting that the amidine protecting groups do not alter the repetitive yield. When the purified products were digested with *Eco*RI (Figure 3), essentially complete (greater than 98%) digestion was observed with samples prepared from either amidine- or amide-protected deoxynucleosides. The small amount of undigested material found in each sample was probably due to hairpin structures (not degraded by this enzyme) which result from the palindromic sequence of this decanucleotide. Amidine-protected deoxynucleoside phosphoramidites have now been used to synthesize various sequence-modified λ P_R promoters and cro gene fragments having up to 57 mononucleotides per segment for studies in vitro and in vivo.[30]

Depurination Studies. Depurination of a polymer-linked deoxyoligonucleotide has been shown to be most severe for a terminal 3′-adenine or guanine.[11] Therefore the depurination rates of protected deoxynucleosides covalently attached through the 3′-hydroxyl to silica gel were studied by using DCA and TFA. As the purine was liberated from the support during depurination its ultraviolet absorbance (A) was monitored spectrophotometrically. A plot of log ($A_{t\to\infty} - A_t$) with respect to time (t) indicated pseudo-first-order decay kinetics for protected deoxyadenosine (Figure 4) and deoxyguanosine (Figure 5). Half-life values determined by this assay are summarized in Table II. These data demonstrate that the amidine derivatives of deoxyadenosine are about 20 times more stable toward depurination with DCA than *N*-benzoyldeoxyadenosine when attached to a polymeric support. Additional depurination studies completed with TFA also show that amidines stabilize deoxyadenosine more toward depurination than does the benzoyl group. However, the results also show that

(30) Dubendorff, J.; Tang, J.-Y.; McBride, L. J.; Beltman, J.; Caruthers, M. H., unpublished results.

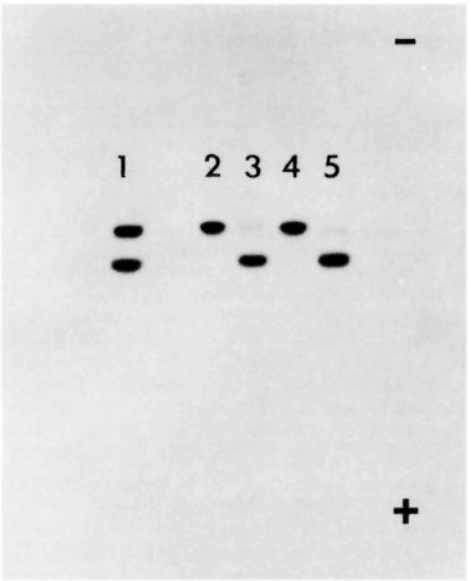


Figure 3. *Eco*RI digestions of gel-purified preparations of [5′-^{32}P]d(pG-G-G-A-A-T-T-C-C-C). Polyacrylamide containing the deoxydecanucleotides (gel shown in Figure 2) was cut from the gel and the DNA extracted by using 10 mM Tris pH 8, 1 mM EDTA. For each sample, the extract was washed with 1-butanol (4×), loaded onto a DE-52 anion-exchange resin, and washed with 0.1 M NaCl, and the DNA was eluted with 2 × 0.1 mL of 1.0 M NaCl. The effluent was diluted to 1.5 mL with absolute ethanol and the DNA isolated by precipitation at −70 °C. *Eco*RI (10 units) in 20 μL of buffer (50 mM NaCl, 10 mM Tris, 10 mM $MgCl_2$; pH 7.6) was added in two equal portions at 16-h intervals. Lanes 2 and 3 (undigested and digested, respectively) are from DNA prepared with amidine-protected deoxynucleotides (Figure 2, lane C) while lanes 4 and 5 (undigested and digested, respectively) are from DNA prepared with amide-protected deoxynucleotides (Figure 2, lane D). Lane 1 is a mixture of the DNA samples loaded into lanes 2 and 3.

Table II. Depurination Half-Lives, $t_{1/2}$, at Room Temperature for Various Exocyclic Amino-Protected Deoxynucleosides Attached to Fractosil-500[a]

compound	$t_{1/2}$, h[b]	$t_{1/2}$, min[c]
d(bzA)	1.7	23
d(acaA)	34	153
d(pyaA)		128
d(dnbA)	30	119
d(ibG)	23	
d(dmfG)	30	
d(dnbG)	21	
d(acaG)	11 (12)[d]	

[a] Attachment to Fractosil-500 was through the 3′-hydroxyl by use of published procedures.[2f] [b] DCA/CH_2Cl_2 (1:50, v/v). [c] TFA/CH_2Cl_2 (1:50, v/v). [d] Controlled-pore glass instead of Fractosil-500.

stronger acids such as TFA decrease the difference in depurination rates between amide- and amidine-protected deoxyadenosine. In contrast, amidine and isobutyryl derivatives of deoxyguanosine have comparable stability toward depurination. The half-life for depurination of **11b** (21 h) was approximately the same as found for *N*-isobutyryldeoxyguanosine (d(ibG)) (23 h).

Compound **5c** and *N*-benzoyldeoxyadenosine were also incorporated into deoxyoligonucleotides and tested for depurination. Thus samples of d(T-T-T-T-T-T-T-T-T-T-A-T-T-T-T-T-T-T-T-T-T) were synthesized on controlled-pore glass by using **1d** and either **8c** or **1a**. These deoxyoligonucleotides still attached to the support and carrying base protecting groups on adenine were treated with 3% trichloroacetic acid for variable times to cause depurination. Following removal of the methyl phosphate protecting groups, the products were freed from the support and deoxyadenosine protecting groups with concentrated ammonium hydroxide, labeled with [^{32}P]phosphate by using T4 kinase and [γ-^{32}P]ATP, and analyzed by polyacrylamide gel electrophoresis.

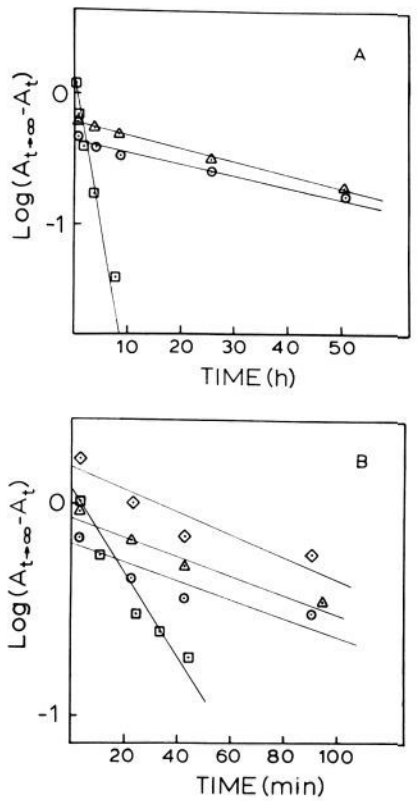


Figure 4. Depurination kinetics of 6-N-protected deoxyadenosines attached covalently to Fractosil-500 or CPG. Panel A, DCA/CH_2Cl_2 (1:50, v/v), panel B, TFA/CH_2Cl_2 (1:50, v/v). □, 6-*N*-Benzoyl-2′-deoxyadenosine; ○, **5c**; △, **5b**; ◇, **5d**.

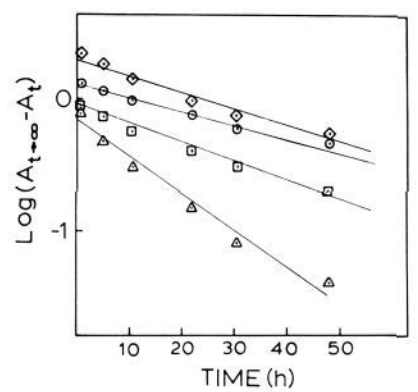


Figure 5. Depurination kinetics of 2-N-protected deoxyguanosines attached covalently to Fractosil-500 or CPG. ◇, 2-*N*-Isobutyryl-2′-deoxyguanosine; ○, **11a**; □, **11b**; △, **11c**. Depurination studies were completed in DCA/CH_2Cl_2 (1:50, v/v).

The depurinated deoxyoligonucleotide which was cleaved at the depurination site by concentrated ammonium hydroxide[6] is visible as a band of radioactivity on a polyacrylamide gel having mobility similar to [5′-^{32}P]d(pG-G-G-A-A-T-T-C-C-C). Analysis of the product and depurinated product after either 4 or 36 h suggests that the depurinated product was most intense in lanes containing deoxyoligonucleotides prepared with *N*-benzoyldeoxyadenosine. This conclusion was confirmed by quantitatively measuring the radioactivity in these bands (Figure 6). When *N*-benzoyldeoxyadenosine was part of the deoxyoligonucleotide, 18% and 50% of the product had depurinated after 4 and 36 h of exposure, respectively, to TCA. In contrast, when *N*,*N*-dimethylacetamidine was the protecting group on deoxyadenosine, no greater than 5% depurination with TCA was observed after 36 h. These results support the depurination studies outlined in Figure 4 and demonstrate the utility of using amidine-protected deoxyadenosine

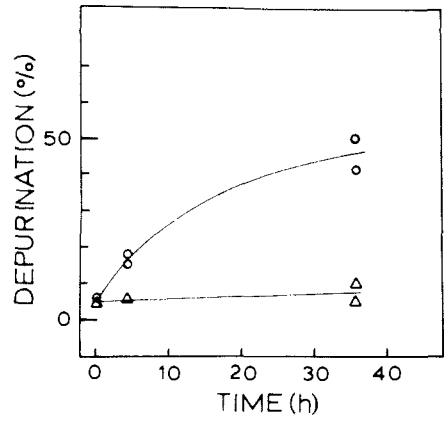


Figure 6. Depurination kinetics of 6-N-protected adenine contained within deoxyoligothymidine attached covalently to CPG. See Experimental Section for details leading to the results shown in this figure. O, d(T-T-T-T-T-T-T-T-T-T-T-bzA-T-T-T-T-T-T-T-T-T-T)-Ⓟ; Δ, d(T-T-T-T-T-T-T-T-T-T-acaA-T-T-T-T-T-T-T-T-T-T)-Ⓟ.

Table III. Deprotection of Amidine Derivatives of Deoxynucleosides

compound	deprotection conditions[a]		
	1[b]	2[c]	3[d]
d(acaA)	3 h	<10 min	10 min
d(dnbA)	4 h	<10 min	10 min
d(pyaA)	24 h	30 min	1 h
d(dmfA)	10 min	<10 min	<10 min
d(bzA)	30 min	30 min	<10 min
d(dnbG)	15 min	20 min	<10 min
d(acaG)	30 min	10 min	15 min
d(pyaG)	5 h	4 h	1 h
d(dmfG)	10 min	<10 min	<10 min
d(ibG)	30 min	1 h	30 min
d(pyaC)	<10 min	10 min	<10 min
d(dnbC)	<10 min	<10 min	<10 min
d(bzC)	<10 min	20 min	<10 min

[a] Results are expressed as half-life measurements. [b] Concentrated NH_4OH (50 °C). [c] Ethylenediamine/phenol/H_2O (40 °C). [d] NH_4OAc/concentrated NH_4OH (50 °C).

within deoxyoligonucleotides where depurination can also be a serious, yield-limiting step with amide-protected deoxyadenosine.[6]

Removal of Amidine Protecting Groups. In order to be useful in DNA synthesis, amidine protecting groups must be removed quantitatively by using extremely mild conditions. We have observed that this was indeed the case (Table III). By use of concentrated ammonium hydroxide at 50 °C, several amidine protecting groups [d(acaA), d(dmfA), d(dnbG), d(acaG), d(dmfG), d(pyaC), d(dnbC)] were removed under conditions comparable to those usually employed for removal of amide protecting groups (10–24 h). As was found earlier with formamidine derivatives of deoxyadenosine,[16b] the addition of ammonium acetate to ammonium hydroxide greatly enhances the deprotection rate for amidine derivatives of deoxyadenosine and deoxyguanosine (Table III). For example, the half-life for d(acaA) was reduced from 2.5 h to 10 min. This was also generally the case with an ethylenediamine/phenol/water solution at 40 °C,[16a] where rates were comparable to those obtained with ammonium hydroxide containing ammonium acetate at 50 °C and 10–30 times faster than with aqueous ammonium hydroxide alone at 50 °C. Deprotection rates with amidine derivatives of deoxycytidine were very fast with all three reagents.

Unlike amide derivatives of deoxycytidine,[19] amidine-protected deoxycytidines or cytidine were stable toward the formation of side products during deprotection with primary amines (Table IV). These side products, 4-*N*-alkylcytidine or 4-*N*-alkyldeoxycytidine, result from nucleophilic attack of a primary amine on C-4 of cytosine rather than on the amide carbonyl. Treatment of **17a** with *n*-butylamine/methanol/*p*-dioxane (1:1:2) at 37 °C showed no detectable side products using conditions which completely removed the amidine protecting group (15 h). In contrast, by use of similar deprotection conditions which lead to the complete removal of various amide protecting groups (Table IV), DMTd(acC), DMTd(ibC), DMTbzC (2′-thp), and DMTd(anC) were observed to yield 3–20% of a side product (4-*N*-*n*-butylcytidine or 4-*N*-*n*-butyldeoxycytidine as confirmed by ^1H NMR spectroscopy). These observations may prove to be especially important where primary amines form an integral part of synthesis or deprotection procedures. Moreover these results suggest that amidines are the preferred protecting group for protection of 4-^{15}N-enriched deoxycytidine (currently underway in this laboratory). Loss of this nuclide from amidine-protected deoxycytidine should not occur during the ammonia deprotection procedure.

Table IV. Studies on the Reaction of N-Protected Cytidine and 2′-Deoxycytidine Derivatives with *n*-Butylamine[a]

compound[b]	$t_{1/2}$, h	$t_{complete}$, h	side product, %[c]
DMTbzC (2′-thp)	1.5	12	15
DMTd(anC)	4.0	24	20
DMTd(ibC)	4.0	24	5
DMTd(acC)	0.2	2	3
DMTd(pyaC) (**17a**)	2.0	15	0

[a] Each compound was treated with *n*-butylamine/methanol/*p*-dioxane (1:1:2, v/v/v) at 37 °C. Analysis was by silica gel TLC using solvent B followed by visualization with HCl (gas). [b] DMTbzC (2′-thp) = 4-*N*-benzoyl-5′-(bis(*p*-anisylphenyl)methyl)-2′-tetrahydropyranylcytidine; DMTd(anC) = 4-*N*-anisoyl-5′-(bis-(*p*-anisylphenyl)-methyl)-2′-deoxycytidine; DMTd(ibC) = 4-*N*-isobutyryl-5′-(bis-(*p*-anisylphenyl)methyl)-2′-deoxycytidine; DMTd(acC) = 4-*N*-acetyl-5′-(bis-(*p*-anisylphenyl)methyl)-2′-deoxycytidine. [c] 0% indicates that no side product was detected by TLC analysis. Estimates of side product amounts were after complete removal of the 4-amino protecting group.

Conclusions

On the basis of these results, deoxynucleoside amidines converted to 3′-phosphoramidites (**8c**, **13b**,**c**, and **18**) are currently being used to synthesize deoxyoligonucleotides. These synthons are preferred because of their enhanced stability toward acid (adenine) and ring substitution (cytosine). Various amidine protecting groups on guanine appear to generally be equivalent chemically to the isobutyrylamide (deprotection, depurination rates). The only real advantage so far observed for guanine amidines is their ease of preparation. When coupled with the in situ approach for DNA synthesis, the one-flask procedure for synthesizing completely protected deoxynucleosides also provides a high-yield method for incorporating analogues into DNA sequences.

Registry No. **1a**, 84416-82-0; **1b**, 84416-84-2; **1c**, 84416-83-1; **1d**, 84416-85-3; **3**, 958-09-8; **4a**, 4637-24-5; **4b**, 19449-30-0; **4c**, 18871-66-4; **4d**, 39650-82-3; **5a**, 17331-12-3; **5b**, 89128-69-8; **5c**, 98532-92-4; **5d**, 88010-85-9; **7c**, 98566-67-7; **7d**, 88010-86-0; **8c**, 98532-93-5; **9**, 961-07-9; **10a**, 1188-33-6; **10b**, 35534-34-0; **10c**, 19429-85-7; **10d**, 826-41-5; **11a**, 17331-13-4; **11b**, 98532-98-0; **11c**, 98532-99-1; **11d**, 98533-00-7; **12b**, 98533-01-8; **12c**, 98533-02-9; **13b**, 100858-48-8; **13c**, 100858-45-5; **14**, 5132-79-6; **15a**, 951-77-9; **15c**, 838-07-3; **16b**, 98533-10-9; **16d**, 98533-08-5; **17a**, 98533-06-3; **17c**, 100858-44-4; **18** (diastereomer 1), 100858-46-6; **18** (diastereomer 2), 100858-47-7; d(G-G-G-A-A-T-T-C-C-C), 93183-37-0; d(T-T-T-T-T-T-T-T-T-T-bzA-T-T-T-T-T-T-T-T-T-T), 100927-29-5; d(T-T-T-T-T-T-T-T-T-T-TacaA-T-T-T-T-T-T-T-T-T-T), 100927-28-4; d(bzA), 4546-72-9; d(ibG), 68892-42-2; d(bzC), 4836-13-9; DMTbzC(2′-thp), 69359-38-2; DMTd(anC), 68892-40-0; DMTd(ibC), 100898-62-2; DMTd(acC), 100898-63-3; *N*-methyl-2-pyrrolidinone, 872-50-4; di-*n*-butylamine, 111-92-2; *n*-butylamine, 109-73-9.