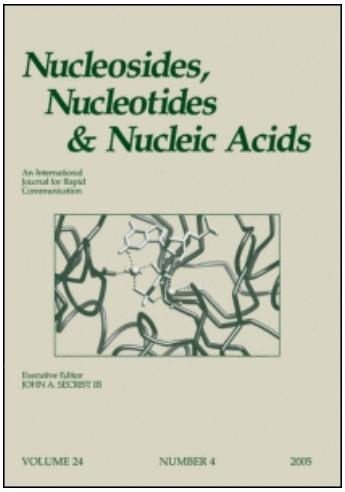

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N-6-Dialkylformamidine-2'-deoxyadenosine Phosphoramidites in Oligodeoxynucleotide Synthesis Raped Deprotection of Oligodeoxynucleotides

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N-6-DIALKYLFORMAMIDINE-2'-DEOXYADENOSINE PHOSPHORAMIDITES IN OLIGODEOXYNUCLEOTIDE SYNTHESIS. RAPID DEPROTECTION OF OLIGODEOXYNUCLEOTIDES.

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Abstract: A series of dialkylformamidine protected deoxyadenosine phosphoramidites were prepared for automated, solid-support DNA synthesis. The set of A^{bz}, G^{dmf}, C^{bz}, T phosphoramidites gave high purity and high yield oligodeoxynucleotides, with complete deprotection at 65 °C in one hour. Different times and temperatures of exposure to concentrated ammonium hydroxide were examined to establish the optimum conditions for deprotection of oligodeoxynucleotides.

Millions of oligodeoxynucleotides are produced on automated synthesizers in thousands of laboratories each year with phosphoramidite nucleosides on solid supports. PCR, DNA sequencing, and probe assays demand more rapid methods for synthesis, deprotection, analysis, and purification. Rapid deprotection of synthetic oligodeoxynucleotides has been pursued with more labile protecting groups,[1] alternative deprotection reagents,[2] and elevated temperatures.[3] After synthesis is complete, the oligodeoxynucleotide is cleaved from the solid support. Protecting groups from the phosphates and the exocyclic amines of the nucleobases are removed, usually with concentrated ammonium hydroxide at 55 °C. The rate limiting operation is the deprotection of the exocyclic amines of the bases when the classical acyl groups are used.[4] By far the slowest species to deprotect is the isobutyryl group from deoxyguanosine which requires 8 hours.[1] Based on the earlier work of Smrt and Holy,[5] and others,[6] we introduced dimethylformamidine (dmf) protected deoxyadenosine **1** and deoxyguanosine **2** phosphoramidites (R = NMe_2) for the automated synthesis of oligodeoxynucleotides (Figure 1).[7] The exocyclic amines of nucleosides react selectively with dialkylformamide- and dialkylacetamide-dimethyl acetals, with no requisite protection of the 3' or 5' hydroxyls. Greater resistance to depurination, rapid deprotection, and ease of monomer preparation are advantages of formamidine protection, relative to the classical acyl protected nucleosides, benzoyl (bz) deoxyadenosine and isobutyryl (ibu) deoxyguanosine.[4]

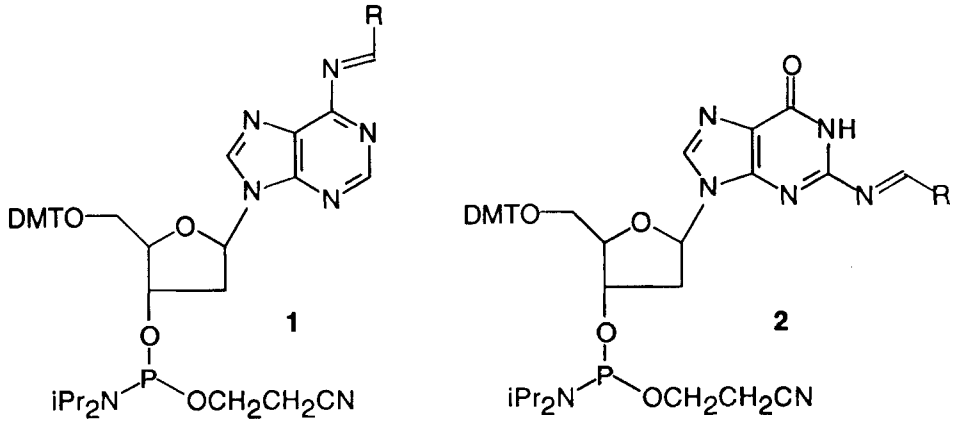

Figure 1. Dialkylformamidine deoxyadenosine **1** and deoxyguanosine **2** cyanoethyl phosphoramidites

Synthesis of primer length oligodeoxynucleotides (<35mers) is efficient. However, the dmf protecting group of deoxyadenosine proved to be unstable to oligodeoxynucleotide synthesis conditions.[5,6] Based on our experience, approximately 5-10% of deoxyadenosine nucleobases lose the dmf group during the normal conditions of automated synthesis. Subsequent phosphitylation of the exocyclic amine leads to higher-molecular weight "branched" oligodeoxynucleotide impurities.[8] These species are evident as slower migrating bands on PAGE and later eluting peaks by reverse-phase HPLC and MicroGel capillary electrophoresis. The loss of the protecting group becomes particularly acute during the synthesis of longer sequences. In this report, a series of formamidine protecting groups has been synthesized with the intention of improving stability during oligodeoxynucleotide synthesis conditions, while retaining rapid deprotection upon treatment with concentrated ammonium hydroxide. Though the ideal formamidine group for deoxyadenosine was not discovered, an alternative set of phosphoramidites gave rapid deprotection without the branched impurities.

A number of dialkylformamidine protecting groups for the exocyclic amines of deoxynucleosides have been reported, including the diethyl, diisopropyl, diisobutyl, and di-n-butyl formamidine derivatives and the dimethylaminoethylene (from N,N-dimethylacetamide) and N-methylpyrrolidin-2-yl (from N-methyl-2-pyrrolidone).[9] However, all of the formamidines mentioned above do not undergo rapid enough removal from the oligodeoxynucleotide by concentrated ammonium hydroxide to qualify as useful alternatives to dimethyl (dmf). We applied a number of new formamidine protecting groups on deoxyadenosine phosphoramidites to confer rapid deprotection kinetics and impart enhanced stability during oligodeoxynucleotide synthesis.

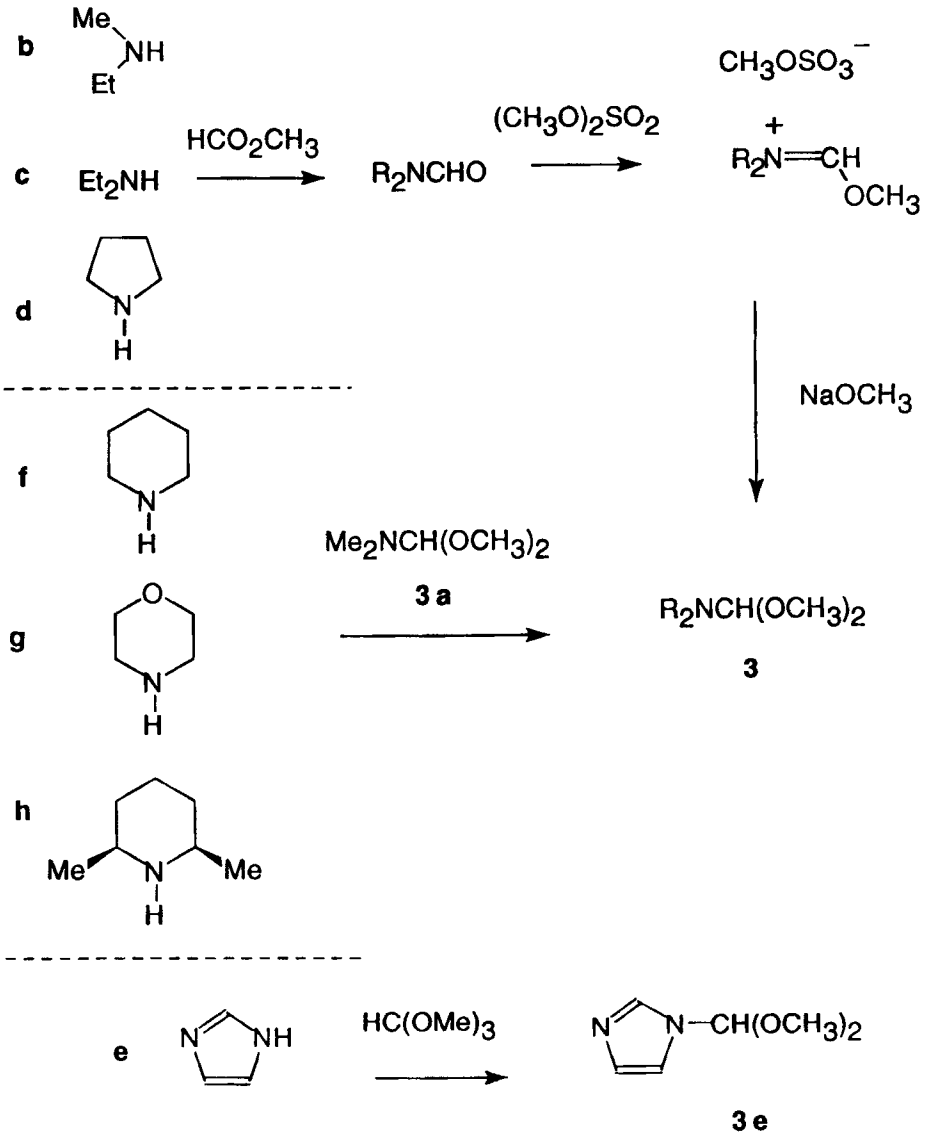

Figure 2. Synthesis of dialkylformamide dimethylacetals **3**

The dialkylformamide dimethylacetals **3** were prepared (Figure 2) by one of three methods:

- Thermal exchange of the corresponding secondary amine and an excess of N,N dimethylformamide dimethylacetal.[10]

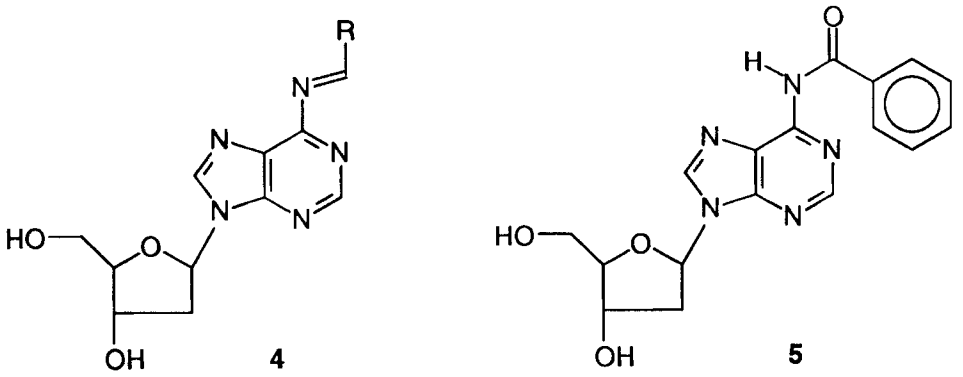

Figure 3. N-6 formamidine **4** and benzoyl **5** 2'-deoxyadenosine

- Methylation of the corresponding formamide (commercially available or synthesized from the secondary amine and methyl formate) with dimethyl sulfate followed by decomposition of the intermediate salt with sodium methoxide.[10,11]
- Acid catalyzed exchange between imidazole and trimethylorthoformate.[12]

Dialkylformamide dimethylacetals **3** react selectively in methanol at N-6 of deoxyadenosine to produce the N-6 dialkylformamidine deoxyadenosine **4** (Figure 3). Reactions proceeded at very different rates at room temperature and could be conveniently monitored by HPLC or TLC. Simple evaporative removal of most of the volatiles caused precipitation of the product in a highly pure state. Relative deprotection rates of the N-dialkylformamidine protected deoxyadenosines were compared with N-benzoyl deoxyadenosine **5** in a 1:1 mixture (v:v) of acetonitrile and concentrated ammonium hydroxide at room temperature (23 °C) and 55 °C (Table 1). The presence of acetonitrile was required to ensure complete solubility of reactants and products. The extent of deprotection at various time points was determined by reverse phase HPLC analysis, monitoring the decrease of protected nucleoside and the increase of deoxyadenosine. From the results, it is seen that a simple correlation between steric bulk of the alkyl substitutents of the formamidine and rate of deprotection does not exist. Larger analogs of dmf **4a**; ethylmethylamino **4b**, and diethylamino **4c**, were far slower to deprotect, in accord with di-n-butylformamidine protected nucleosides.[9] Attention was shifted to cyclic dialkylformamidines. Here, several analogues in the 6-membered ring series **4f, 4g, 4h** showed reasonable deprotection kinetics, more rapid than N-6-benzoyl-2'-deoxyadenosine **5**, and were converted to nucleoside phosphoramidites for study in oligodeoxynucleotide synthesis.

The protected deoxyadenosines **4f, 4g, 4h** were reacted with 4, 4'-dimethoxytrityl chloride (DMTCl) in pyridine to give **6f, 6g, 6h** (Figure 4).

Table 1. Deprotection times for protected deoxyadenosine (10mM) in acetonitrile:conc. ammonia / 1:1, measured by complete disappearance of protected deoxyadenosine. HPLC detection at 254nm was corrected for relative extinction coefficients (e = 15,400 dA and e = 3000 dA^{form} **4a-h**).

	R =	23 °C	55 °C (hours)
4a	dimethylamine (dmf)	6	.75
4b	ethylmethylamine	>24	20
4c	diethylamine	>24	24
4d	pyrrolidine	>24	6
4e	imidazole	<0.1	<0.1
4f	piperidine	9	1.2
4g	morpholine	4	0.5
4h	(cis) 2,6 dimethylmorpholine	8	1.0
5	benzoyl	16	2.0

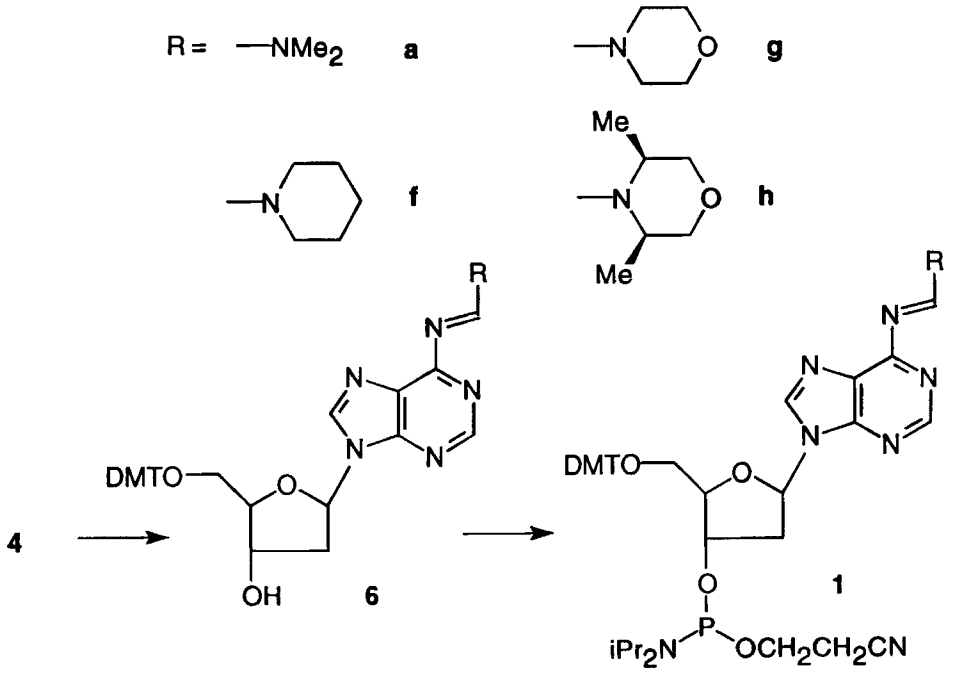

Figure 4. Synthesis of dialkylformamidine deoxyadenosine phosphoramidites **1a, 1f, 1g, 1h**

Alternatively, 5' DMT-2'-deoxyadenosine could be reacted with formamidine acetals **3** to give **6**. Conversion to cyanoethylphosphoramidites **1f, 1g, 1h** was effected with bisdiisopropylamino-cyanoethylphosphine ($(iPr_2N)_2POCH_2CH_2CN$) and diisopropylammonium tetrazolide ($iPr_2NH_2^+$ CHN_4^-) in dichloromethane.[13]

Table 2. Deprotection of A-T dimers by concentrated ammonium hydroxide after one hour, 22°C for cleavage from support (t=0). Measured by integrated product areas of protected and unprotected dimer. HPLC detection at 254nm was corrected for relative extinction coefficients (e = 22, 000 A-T, and e = 12,000 A^{form} - T).

	R =	t = 0	t= 1 hr, 55° C	t = 8 hr, 22° C
1a	dimethylformamidine	91.8%	100%	100%
1f	piperidine	95.1%	100%	100%
1h	cis-2,6 dimethylmorpholine	100%	100%	100%

In a model study of deprotection rates, a dimer A-T was synthesized with phosphoramidites **1a, 1f, 1h** on a 3' T CPG support and deprotected in concentrated ammonium hydroxide. The endpoints of the deprotection reactions were comparable to those determined on the protected monomers (Table 2).[14] Each formamidine protecting group was sufficiently labile for further study.

A series of oligodeoxynucleotides were synthesized with dialkylformamidine **1a,f,g,** and benzoyl **7** dA phosphoramidite monomers in a comparison study under conventional automated synthesis conditions. The 50mer sequence, 5' AGG GCC GAG CGC AGA AGT GGT CCT GCA ACT TTA TCC GCC TCC ATC CAG TG 3', was synthesized separately and concurrently with either **1a, 1f, 1g,** or **7** dA phosphoramidites. The other monomers were G^{dmf}, C^{bz}, and T deoxynucleoside phosphoramidites, a set that affords rapid deprotection. All other parameters were held constant to isolate the effect of the deoxyadenosine protecting group. The synthesis scale was 0.2μmole and average step-wise yield was monitored every fifth base addition by conductivity quantitation of the dimethoxytrityl cation (Table 3).[15] Cleavage from the solid support (1000Å CPG) was conducted for one hour at room temperature, immmediately following synthesis, in concentrated ammonium hydroxide. The supernatant solution was deprotected by heating at 65°C for one hour. The purity and yield of the oligodeoxynucleotides were analyzed by reverse-phase HPLC, polyacrylamide slab gel electrophoresis (PAGE), and MicroGel capillary electrophoresis (CE).[16,17] The CE method provides the highest resolution in the analysis of the four samples (Figure 5). All four syntheses provided reasonable yield and purity of this relatively long, 50mer oligodeoxynucleotide, but the sample prepared with benzoyl dA, **7**, (Fig. 5D) is free of the later eluting impurities that occur in all three formamidine protected samples (Fig. 5A, 5B, 5C). The formamidine protecting groups on deoxyadenosine confer only a modest time savings in deprotection. With dimethylformamidine protected deoxyguanosine, significant time savings is realized compared to the standard, amide set of phosphoramidites (A^{bz}, G^{ibu}, C^{bz}, T). Therefore, the set of A^{bz}, G^{dmf}, C^{bz}, T

Table 3. Comparison of syntheses of a 50mer with **1a**, **1f**, **1g**, or **7** and G^{dmf}, C^{bz}, T phosphoramidites. 1 odu = 1 Abs. unit of a 1 ml solution at 260nm = 33μg DNA. ASWY = average step-wise yield measured by AutoAnalysis conductivity[18]

A phosphoramidite	crude odu	Final ASWY
1a dmf	73	98.8%
1f piperidine	73	98.5%
1g morpholine	76	98.4%
7 benzoyl	63	98.2%

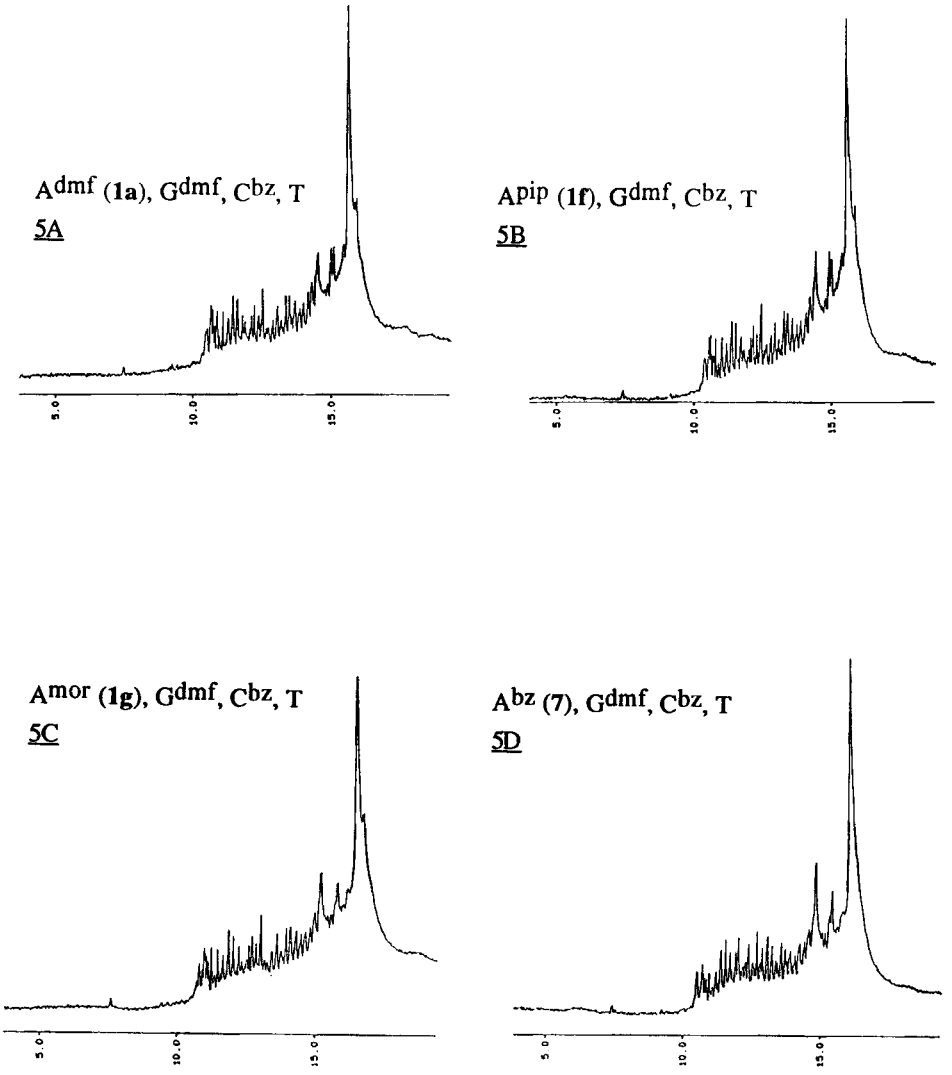

Figure 5. MicroGel capillary electrophoresis analysis of crude 50mers.

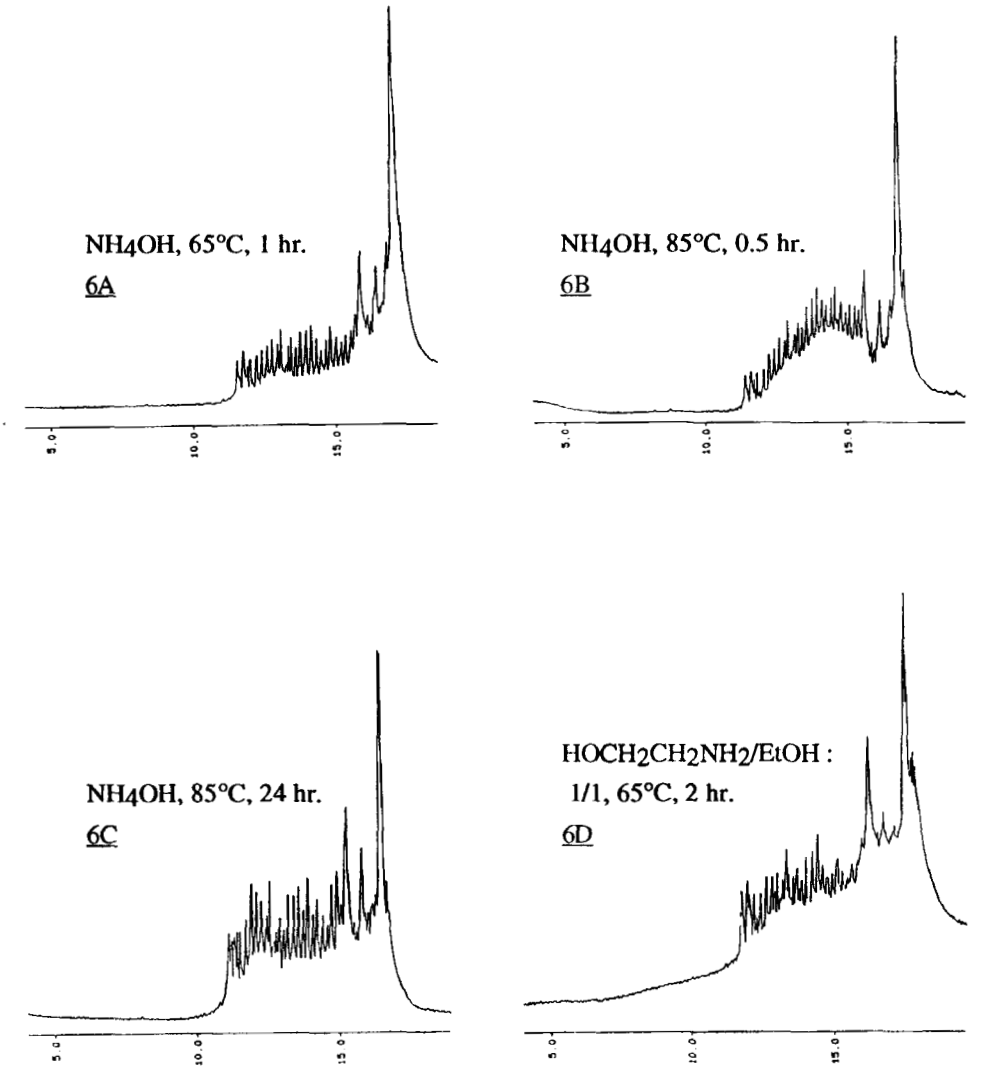

Figure 6. MicroGel capillary electrophoresis analysis of ethanol precipitated 50mers, synthesized with A^{bz} (7), G^{dmf}, C^{bz}, T phosphoramidites.

realizes a very rapid deprotection and provides oligodeoxynucleotides free of the impurities that plague the formamidine protected deoxyadenosines.

Reagents other than concentrated ammonium hydroxide have been reported to be rapid and effective for oligodeoxynucleotide deprotection.[2] An anhydrous mixture of ethanolamine and ethanol (1:1, v:v), has been reported to rapidly cleave and deprotect acyl-protected oligodeoxynucleotides.[2] Increased temperature (>55 °C) also serves the same purpose as alternative deprotection reagents and labile protecting groups.[3] Deprotection rates were measured and compared. A single 50mer sequence was

synthesized with the set of A^{bz}, G^{dmf}, C^{bz}, T phosphoramidites. After synthesis was complete, the support was divided into four aliquots. Deprotection was conducted under four different conditions; treatment in concentrated ammonium hydroxide for 1 hour at 65 °C (Fig. 6A), at 85 °C for a brief (Fig. 6B) and extended period (Fig. 6C), and by the alternative reagent, ethanolamine/ethanol (Fig. 6D). This reagent is viscous and does not flow under normal pressures on automated synthesizers. Also, it is relatively non-volatile, requiring long periods of vacuum centrifugation to concentrate the samples. While a higher temperature (85°C) accelerates deprotection, internucleotide cleavage seems to occur under prolonged treatment (Fig. 6C). The deprotection conditions of concentrated ammonium hydroxide for 1 hour at 65°C gave the most pure product.

Enzymatic digestion of the oligodeoxynucleotides and analysis of the resulting deoxynucleoside mixture by HPLC gives a sensitive and quantitative detection of incomplete deprotection, as well as any nucleoside chemical modifications that occur during synthesis or deprotection.[15,17] Some loss of exocyclic amine protecting groups is incurred during the digest reaction. The two sets of phosphoramidites (A^{bz}, G^{dmf}, C^{bz}, T, and A^{bz}, G^{ibu}, C^{bz}, T), with the sole difference being the G protecting group, were assayed by digestion of the crude oligodeoxynucleotides from Figure 5 and inspection of the HPLC chromatograms (Figure 7). The quantitated base compositions of the two samples were in close agreement with the sequence. There were no significant base modifications or remaining protected deoxynucleosides in either sample.

Conclusion

Altering the formamidine substituents of deoxyadenosine phosphoramidites did not completely banish the uncharacterized impurities that occur during oligodeoxynucleotide synthesis. However, when benzoyl protected deoxyadenosine A^{bz}, is used with G^{dmf}, C^{bz}, and T, deprotection is complete in one hour at 65°C. This set of phosphoramidites realizes the benefit of rapid deprotection and high-purity oligodeoxynucleotide synthesis, at least up to 50mers in length.

Experimental

Oligonucleotides were synthesized on an Applied Biosystems Model 394 DNA Synthesizer at the 0.2 μmole scale. Applied Biosystems cyanoethyl phosphoramidite nucleosides and other reagents were employed with the standard .2 UM CE synthesis cycle and CESS end procedure for automated cleavage.

Deprotection was conducted with 2 ml deprotection solution in 4 ml glass vials with Teflon lined caps. Considerable pressure results from heating enclosed concentrated ammonium hydroxide solutions. Hot vials should be handled behind a shield and padded gloves should be worn. After drying the samples by vacuum centrifugation and dissolution

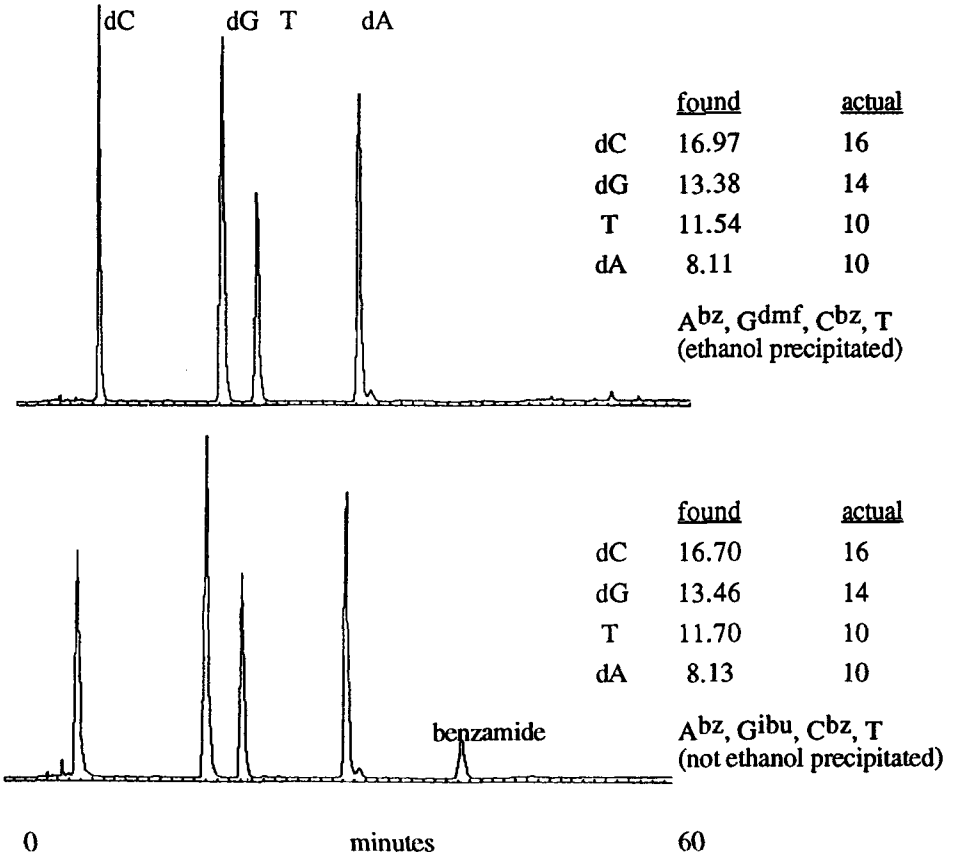

Figure 7. Enzymatic digestion of 50mers synthesized with two sets of phosphoramidites. HPLC analysis of the deoxynucleoside mixtures.

in water (0.1 odu / 100 µl), the deprotected oligodeoxynucleotides were analyzed by capillary electrophoresis on an Applied Biosystems Model 270A.[16,17]

The nucleoside composition of the oligodeoxynucleotides was assessed by digestion of 25 µg (0.8 odu) of the sample with snake venom phosphodiesterase (Pharmacia) and bacterial alkaline phosphatase (Pharmacia) in 15 mM $MgCl_2$, 30 mM Tris, pH 7.5 at 37 °C for 12 hours. The mixture was ethanol precipitated twice and the supernatant was analyzed by HPLC on a reverse-phase cartridge (Aquapore RP-300, 220 x 4.6 mm, Applied Biosystems) using a gradient of mobile phases A: 3% acetonitrile in 0.1M triethylammonium acetate and B: acetonitrile.[19]

NMR were recorded on a Varian Unity 300 FT operating at 295.95 MHz for ^1H and 121.42 MHz for ^{31}P. Chemical shifts are reported in ppm, relative to tetramethylsilane (^1H) and phosphoric acid (^{31}P). Melting points were determined on a MelTemp II and are uncorrected. UV spectra were recorded on an Hewlet Packard 8451A

Diode Array spectrophotometer. Solvents were purchased from Burdick & Jackson, Division of Baxter Diagnostics Inc. and used without further purification. 5'-Dimethoxytrityl-2'-deoxyadenosine and 4, 4'-dimethoxytrityl chloride were purchased from Sigma Chemical Co. Secondary amines were purchased from Aldrich Chemical Co.

N-dimethoxymethyl pyrrolidine **3d**

Pyrrolidine-1-carboxaldehyde (25.0 g, 0.25 mol) and dimethylsulfate (31.8 g, 0.25 mol) were stirred overnight in a 250 mL round-bottom flask under argon. In a separate flask, sodium metal (6.0 g, 0.26 mol) was added in small pieces, slowly to 100 mL of methanol (dried over 3Å molecular sieves) at 0 °C under argon. The ice bath was removed and the mixture was stirred at room temperature for 2 hours until all the sodium had gone into solution. An addition funnel was attached to the flask and the sulfate salt of the carboxaldehyde formed above was added dropwise through the addition funnel to the sodium methoxide. The mixture was stirred overnight at room temperature. A distillation apparatus was attached to the flask and the material was fractionally distilled into a cooled receiver (-78 °C), with all the distillate between 10 - 40 °C collected as one fraction (pot temperature rose from room temperature to 160 °C). The collected distillate was then placed under vacuum on a rotary evaporator to remove methanol. The last traces of solvent were removed under high vacuum to obtain 25.15 g (69% yield) of a clear liquid **3d** (lit. bp 160-161°C at 740 torr[10]). ^{1}H NMR ($CDCl_3$): 4.43 (s, 1H), 3.32 (s, 6H), 2.70 (m, 4H), 1.77 (m, 4H).

N-dimethoxymethyl imidazole **3e**

Imidazole (60.00 g, 0.88 mol), trimethylorthoformate (257.0 g, 2.42 mol), and p-toluenesulfonic acid monohydrate (4.00 g, 20 mmol) were combined in a round-bottom flask equipped with a magnetic stir bar and distillation head. The mixture was stirred and heated at 110 °C for 36 h, until methanol ceased to distill. Excess trimethylorthoformate was removed under vacuum. Sodium bicarbonate (one gram) was added and the mixture was fractionally distilled under vacuum to obtain 96.46 g (77% yield) of a clear liquid **3e**, bp 85 °C at 1.0 mm. ^{1}H NMR ($CDCl_3$): 7.72 (s, 1H), 7.08 (s, 2H), 5.96 (s, 1H), 3.34 (s, 6H).

N-dimethoxymethyl piperidine **3f**

N,N-Dimethylformamidedimethylacetal (86.01 g, 1.00 mol) and piperidine (126.76 g, 1.00 mol) were mixed in a round-bottom flask with a reflux condenser attached. The stirred mixture was heated slowly over a period of 6 hours to 190 °C and held at that temperature for 2 hours. The mixture was cooled and fractionally distilled under vacuum to obtain 110.39 g (69% yield) of a clear liquid **3f**, bp 42 °C at 1.0 mm (lit. 83 °C at 15 mm[10]). ^{1}H NMR ($CDCl_3$): 4.36 (s,1H), 3.32 (s, 6H), 2.58 (m, 4H), 1.45-1.57 (m, 6H).

N-dimethoxymethylmorpholine **3g**

N,N-Dimethylformamidedimethylacetal (125.43 g, 1.00 mol) and morpholine (88.00 g, 1.00 mol) were reacted under the same condtions as **3f** above to obtain 102.16 g (63%) of a clear liquid **3g**, bp 55 °C at 1.0 mm Hg (lit. 87°C at 15 torr[10]). ^{1}H NMR ($CDCl_3$): 4.36 (s, 1H), 3.7 (t, 4H, J = 4.6 Hz), 3.33 (s, 6H), 2.6 (t, 4H, J = 4.6 Hz).

N-6-Piperidinoformamidine-2'-deoxyadenosine **4f**

2'-Deoxyadenosine (15.00 g, 55.7 mmol) was azeotroped to dryness with 100 ml chloroform three times under reduced pressure. Methanol (100 mL, dried over 3Å molecular sieves) and N-dimethoxymethylpiperidine (17.75 g, 111.5 mmol) were added and the mixture was stirred at room temperature for 6 hours under argon. Methanol was removed under reduced pressure. Acetonitrile (100 mL) was added and the suspension was stirred overnight at room temperature. The solid material was filtered, rinsed twice with 50 ml acetonitrile, and subjected to vacuum to give 14.05 g of a white solid **4f** (73% yield): mp 178-180 °C. UV max 312 nm. ^{1}H NMR ($CDCl_3$): 8.98 (s, 1H), 8.48 (s, 1H), 7.96 (s, 1H), 6.70 (d, 1H, J = 4.6 Hz), 6.37 (dd, 1H, J = 5.6, 9.4 Hz), 4.80 (d, 1H, J = 4.8 Hz), 4.24 (s, 1H), 3.93 (m, 3H), 3.75 (t, 1H, J = 12 Hz), 3.57 (m, 3H), 3.07 (m, 1H), 2.4 (dd, 1H, J = 5.0, 5.8 Hz), 1.7 (m, 6H).

N-6-Morpholinoformamidine-2'-deoxyadenosine **4g**

2'-Deoxyadenosine (10.00 g, 37.1 mmol) and N-dimethoxymethylmorpholine **3g** (18.0 g, 111.7 mmol) were reacted under the same conditions as **3f** above to give 13.69 g of a white solid **4g** (79% yield): mp 155-157 °C. UV max 310 nm. ^{1}H NMR ($CDCl_3$): 9.00 (s, 1H), 8.52 (s, 1H), 8.05 (s, 1H), 7.94 (s, 1H), 6.59 (bs, 1H), 6.36 (dd, 1H, J = 3.9,5.6 Hz), 4.82 (d, 1H, J = 4.9 Hz), 4.25 (s, 1H), 4.02 (m, 3H), 3.80 (m, 3H), 3.67 (m, 3H), 3.61 (m, 3H), 3.41 (m, 2H), 3.15 (m, 2H), 2.33 (dd, 2H, J = 5.4, 13.4 Hz).

5' Dimethoxytrityl-N-6-piperidinoformamidine-2'-deoxyadenosine **6f**

5'-Dimethoxytrityl-2'-deoxyadenosine (10.00 g, 18.1 mmol), N-dimethoxymethylpiperidine **3f** (6.9 g, 43.4 mmol), and 25 mL of methanol (dried over 3 Å molecular sieves) were mixed in a round-bottom flask under argon and stirred at room temperature for 3 days. The crude product was diluted with 250 ml EtOAc and washed successively with saturated aqueous $NaHCO_3$ (3 x 100 mL), H_2O (2 x 100 mL), and brine (1 x 100 mL). The organic solution was dried over $MgSO_4$, filtered, and concentrated under vacuum to give 11.51 g of a white solid **6f** (99% yield): mp 121-122 °C. ^{1}H NMR ($CDCl_3$): 8.96 (s, 1H), 8.46 (s, 1H), 8.03 (s, 1H), 7.2-7.4 (m, 9H), 6.77 (d, 4H, J = 8.9 Hz), 6.50 (t, 1H, J= 6.6 Hz), 4.68 (m, 1H), 4.19 (m, 1H), 3.89 (m, 2H), 3.75 (s, 6H), 3.37-3.53 (m, 5H), 2.76 (m, 1H), 2.55 (m, 1H), 1.69 (m, 6H).

5' Dimethoxytrityl-N-6-morpholinoformamidine-2'-deoxyadenosine **6g**

5'-Dimethoxytrityl-2'-deoxyadenosine (7.50 g, 13.5 mmol) and N-dimethoxymethylmorpholine (7.2 g, 43.0 mmol) were reacted under the same conditions as **6f** above to give 8.80 g of a white solid **6g** (99% yield): mp 70-72°C. ^{1}H NMR ($CDCl_3$): 8.97 (s, 1H), 8.49 (s, 1H), 8.05 (s, 1H), 7.19-7.41 (m, 9H), 6.78 (d, 4H, J = 8.9 Hz), 6.50 (t, 1H, J= 6.5 Hz), 4.68 (m, 1H), 4.18 (m, 1H), 3.99 (t, 2H, J = 4.9 Hz), 3.78 (m, 4H), 3.75 (s, 6H), 3.56 (t, 2H, J = 4.8 Hz), 3.39 (m, 2H), 3.05 (bs, 1H), 2.80 (m, 1H), 2.54 (m, 1H).

3' Cyanoethyl-diisopropyl-phosphoramidite-5'-dimethoxytrityl-N-6-piperidinoformamidine-2'-deoxyadenosine **1f**

5'-Dimethoxytrityl-N-6-piperidinoformamidine-2'-deoxyadenosine **6f** (8.00 g, 12.3 mmol) was azeotroped twice with 100 mL CH_2Cl_2. Diisopropylammonium tetrazolide (623 mg, 3.69 mmol), bis-diisopropylamino-cyanoethylphosphite (4.83 g, 16.0 mmol), and 50 mL of CH_2Cl_2 were added and the mixture was stirred for 18 hours at room temperature under argon. The mixture was diluted with 250ml EtOAc and washed successsively with saturated aqueous $NaHCO_3$ (3 x 200 mL), H_2O (2 x 200 mL), and brine (1 x 200 mL). The organic solution was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was dissolved in 20ml of acetonitrile and triturated with hexane (2 x 100 ml). The solid was collected by filtration and subjected to vacuum to give 9.29 g of a white solid **1f** (89% yield). ^{1}H NMR ($CDCl_3$): 8.97 (s, 1H), 8.49 (s, 1H), 8.08 (d, 1H, J = 8.1 Hz), 7.20-7.43 (m, 9H), 6.78 (m, 4H), 6.49 (m, 1H), 4.78 (m, 1H), 4.29 (t, 1H, J = 3.4 Hz), 3.35-3.94 (m, 10H), 3.77 (s, 6H), 2.88 (m, 1H), 2.61 (t, 2H), 1.69 (m, 6H), 1.18 (m, 12H). ^{31}P NMR ($CHCl_3$): 146.57, 146.37 (decoupled).

3' Cyanoethyl-diisopropyl-phosphoramidite-5'-dimethoxytrityl-N-6-morpholinoformamidine-2'-deoxyadenosine **1g**

5'-Dimethoxytrityl-N-6-morpholinoformamidine-2'-deoxyadenosine **6g** (6.70 g, 10.3 mmol) was reacted under the same conditions as **6f** above to give 8.54 g of a white solid **1g** (97% yield). ^{1}H NMR ($CDCl_3$): 8.98 (s, 1H), 8.51 (s, 1H), 8.1 (d, 1H, J = 7.9 Hz), 7.19-7.42 (m, 9H), 6.80 (m, 4H), 6.50 (t, 1H, J = 5.5 Hz), 4.77 (m, 1H), 4.29 (m, 1H), 4.00 (t, 2H, J = 4.8 Hz), 3.34-3.85 (m, 13H), 3.77 (s, 6H), 2.90 (m, 2H), 2.62 (t, 1H, J = 6.3 Hz), 2.46 (t, 1H, J = 6.45), 1.19 (m, 12H). ^{31}P NMR ($CHCl_3$): 146.58, 146.40 (decoupled).

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