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Synthesis of 4-triazolopyrimidinone nucleotide and its application in synthesis of 5-methylcytosine-containing oligodeoxyribonucleotides

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ABSTRACT

5'-O-Dimethoxytritylthymidine (2) was phosphorylated and base-modified simultaneously to yield the 4-triazolopyrimidinone nucleotide (3). Coupling between (3) and other common deoxyribonucleotides gave a fully protected nonamer (4). Deblocking under different conditions yielded the nonamer as phosphodiester with concomitant conversion of 4-triazolopyrimidinone to 5-methylcytosine (aqueous ammonia) or thymine (N^1,N^1,N^3,N^3-tetramethyl - guanidinium syn-4-nitrobenzaldoximate solution).

INTRODUCTION

5-Methyldeoxycytidine (m^5C)(1), whose role in the eukaryotic gene has been the subject of intense speculation,[2-6] is a mutation "hot spot" for the C:G→T:A transition in the DNA of E. coli.[7,8] Significance of this substitution has been demonstrated in the expression of the lac repressor gene (lac I), which has a very weak promotor. Through this single base substitution of C:G→T:A at position -35 (Figure 1), a 10-fold enhancement in I expression was achieved.[8]

An attractive mechanism for this transition of C:G→m^5C:G→T:A has been proposed by Coulondre.[7] 5-Methylcytosine (m^5C) probably yields thymine (T) through deamination. The resulting T:G base pair would then undergo a mismatch repair leading to the base mutation T:A.

In order to investigate this transition m^5C→T in DNA, I carried out the synthesis of the nonanucleotide (underlined in Figure 1) at the -35 region of the lac I gene with the "hot spot" (*) in both its methylated (HO-$^{5'}$ATGG$m^5\overset{*}{C}$GCAA$^{3'}$-OH) and mutated (HO-$^{5'}$ATGG$\overset{*}{T}$GCAA$^{3'}$-OH) states. Although this can be achieved through two separate syntheses via the established approaches,[9-11] a modified procedure described in this paper proved more efficient. In this new approach, I first synthesized a fully protected nonanucleotide ATGG($\overset{*}{H}$)GCAA with a newly prepared 4-triazolopyrimidinone

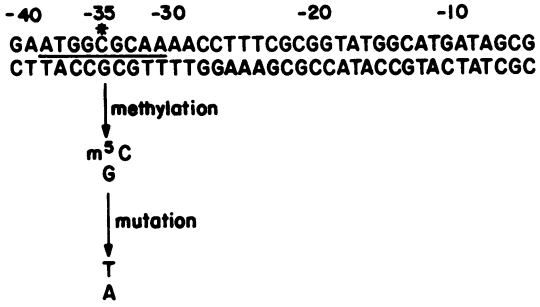

Fig. 1. Sequence of promotor of lac I gene.

nucleotide (H) as the "hot spot" via the phosphotriester method. Subsequent deprotection under different conditions yielded the nonamer in its methylated (ATGGm^{5}CGCAA) or mutated (ATGGTGCAA) state.

RESULTS AND DISCUSSION

Since the methodology for converting the three common deoxyribonucleotides (A,G,T) to 5'-O-dimethoxytrityl-3'-(p-chlorophenyl cyanoethyl phosphotriester) for subsequent condensation has already been well established,[11] the first objective of this work, therefore, remains in the synthesis of the m^{5}C nucleotide.

Recently, in our study of phosphorylation of ribonucleosides, modification of uridine was observed. The uracil residue was transformed in the presence of 1,2,3,4-tetrazole and p-chlorophenyl phosphodichloridate into 4-tetrazolopyrimidinone, that was subsequently converted to cytosine by ammonia under mild conditions.[12] Similar modification of uridine effected by 1-(mesitylene-2-sulfonyl)-tetrazolide (Ms-Te) has also been reported by Reese and Ubasawa.[13] Via the same conversion route, thymidine, having the general feature of 5-methylpyrimidinone, should theorectically yield 5-methyldeoxycytidine.

With this consideration, a new strategy was envisaged in which 5'-O-dimethoxytritylthymidine (2) could be converted into a fully protected 4-triazolopyrimidinone nucleotide H(3) in a single reaction (triazole/p-chlorophenyl phosphodichloridate) (Figure 2). Coupling of (3) with other common nucleotides (A,C,G,T) would yield H-containing oligonucleotides (4), which, via ammonia treatment at a later stage, could undergo the final deblocking and the concomitant conversion of 4-triazolopyrimidinone to give

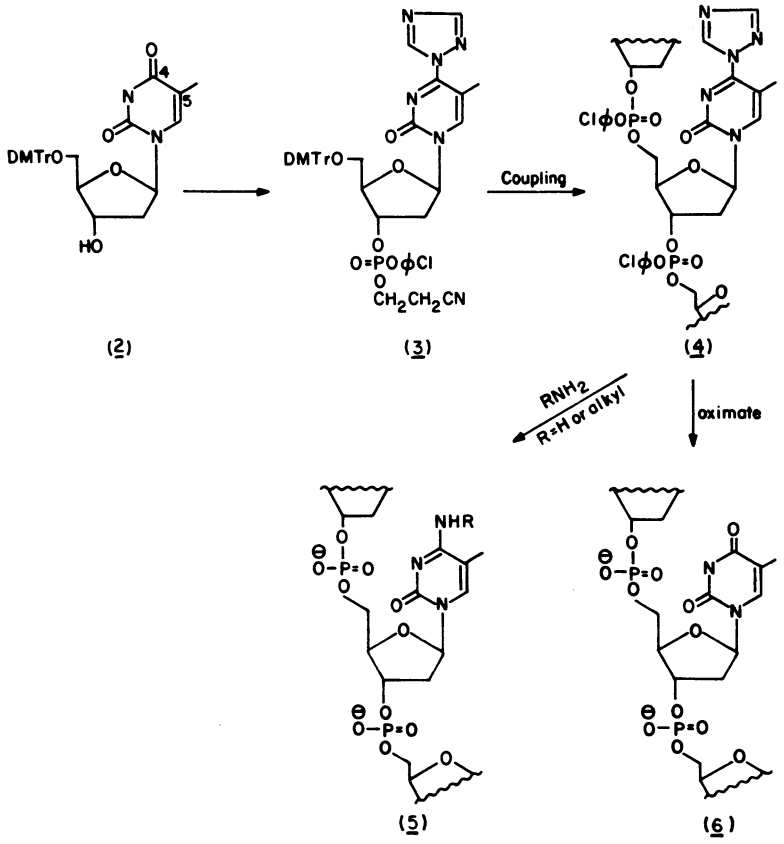

Fig. 2. Strategy for the synthesis of thymine- and 5- methylcytosine-containing oligonucleotides.

the 5-methylcytosine-containing nonamer (5).

In a study of the effect of oximate ions on 4-tetrazolopyrimidinone, Reese and Ubasawa reported regeneration of uracil residue.[13] Therefore, in the present study, N^1,N^1,N^3,N^3-tetramethylguanidinium syn-4-nitrobenzaldoximate, as an alternative deblocking agent to ammonia,[14] should be able to convert nonaoligonucleotide (4) to (6) with transformation of the 4-triazolopyrimidinone residue to thymine.

Obviously, feasibility of this new scheme depends on:

i) capability of 1,2,4-triazole/p-chlorophenyl phosphodichloridate to modify the thymine moiety which, unlike uracil, has been shown to resist analogous

modification attempt by 1-(mesitylene-2-sulfonyl)-tetrazole,[13]
ii) stability of the 4-triazolo group of the pyrimidinone in sterically hindered amines which would be required for converting (3) to phosphdiester for subsequent synthesis,
iii) reactivity of oxymate towards 5-methyl-4-triazolopyrimidinone in the deoxy-series. A series of tests were conducted as a model study (Figure 3).

5'-Dimethoxytritylthymidine (2) was silylated to yield the 3'-(tert-butyldimethylsilyl)-thymidine (7),[15] which was then treated with p-chlorophenyl phosphodichloridate and 1,2,4-triazole in pyridine for 4 days to give the 4-triazolopyrimidinone derivative (8). Both nmr (2 × 1H, singlets at δ9.30 and 8.43) and uv (327 nm) of (8) suggested the structure designated.[13]

Ammonia treatment of (8) in dioxan afforded the 5-methyldeoxycytidine

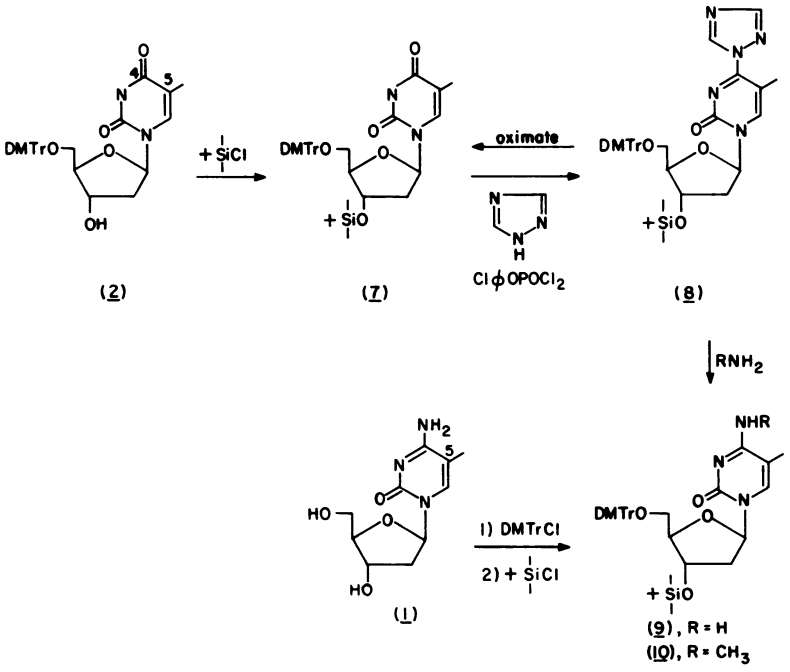

Fig. 3. Chemical Conversion of 5'-O-Dimethoxytritylthymidine to 5-Methyl-2'-deoxycytidine derivatives.

derivative (9),[13] which was identical with a sample prepared from 5-methyldeoxycytidine (1) via tritylation and silylation.[15] Treatment of (8) with methylamine readily yielded the N^4-methyldeoxycytidine derivative (10). However, in the more sterically hindered amines, such as diethylamine, diisopropylamine and triethylamine, the 4-triazolo group of (8) remained stable even after a long period (7 days). In another test of the triazolo group, (8) was treated with N^1,N^1,N^3,N^3-tetramethylguanidinium syn-4-nitrobenzaldoximate. The thymidine derivative (7) was regenerated.[13]

5'-O-Dimethoxytritylthymidine (2) was treated with an excessive amount of p-chlorophenyl phosphodichloridate (3 mol eq) and 1,2,4-triazole (6 mol eq) in pyridine for 4 days to give the base-modified phosphodiester (Figure 4). Addition of β-cyanoethanol quickly yielded the desired phosphodiester H (3). As expected, with a shorter interval in the phosphodiester formation (5 min), the same procedure yielded only the unmodified thymidine nucleotide (11) (Table I), which can be further modified into (3) by triazole and p-chlorophenyl phosphodichloridate.

When triazole was substituted with 1,2,3,4-tetrazole, the rate of the base modification was greatly increased, yielding the 4-tetrazolopyrimidinone nucleotide (12). However, unlike trizaole, tetrazole, even in controlled amounts and for very short reaction times, could not be used soley for 3-phosphorylation without modifying the thymine residue.

Data in Table I, therefore, indicated that the selection of azole reagents and the control of reaction time are crucial for avoiding any unwanted modification during 3'-phosphorylation of thymidine.

As for the present study, the direct route of (2) to (3) was adopted for the subsequent synthesis. Stability of the 4-triazolo group of the pyrimidinone in acid was proved during the detritylation of (3). In 2% benzenesulfonic acid (methanol/dichloromethane, 3:7 v/v),[11] the 5-hydroxyl nucleotide was obtained without any alteration in the base residue.

Coupling between protected nucleotides (A,C,G,T,H) to form nonamer ATGG($\overset{*}{H}$)GCAA was accomplished by the well-established three-step approach: (i) decyanoethylation of one nucleotide block, (ii) detritylation of another, (iii) condensation of the two blocks in the presence of 1-(mesitylene-2-sulfonyl)-tetrazole.[11] The strategy and relevant data of the synthesis are presented in Table II.

The fully protected nonamer ATGG($\overset{*}{H}$)GCAA was then detritylated with the 2% benzenesulfonic acid and was further deprotected under two different conditions.[11] In aqueous ammonia, the nonamer yielded the phosphodiester

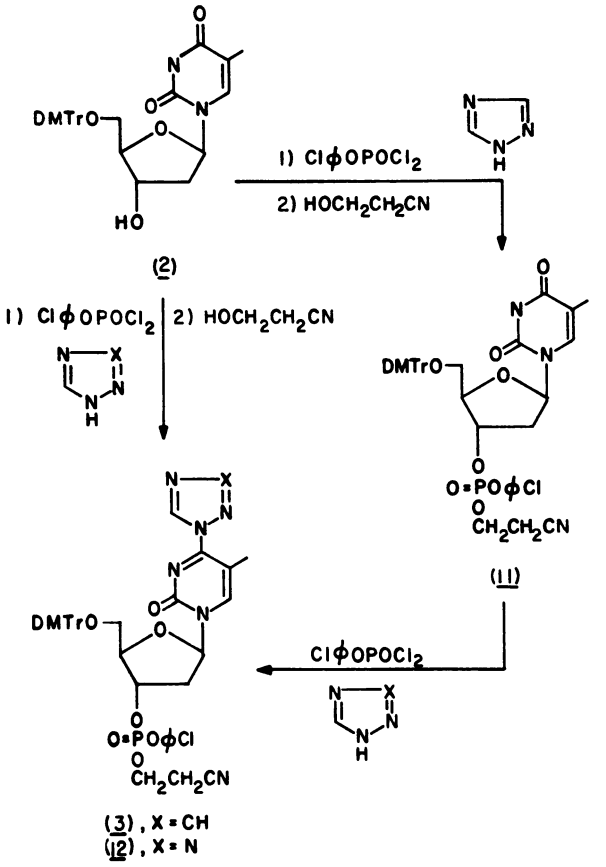

Fig. 4. Synthesis of 4-azolopyrimidinone deoxyribonucleotides (3) and (12).

ATGGm^{5}C*GCAA. In N^1,N^1,N^3,N^3-tetramethylguanidinium syn-4-nitrobenzaldoximate solution.[14] the nonamer ATGGT*GCAA was obtained.

Both deprotected fragments were then purified by PEI tlc. The isolated samples were labeled with ^{32}P at the 5'-position using [$\gamma^{32}P$]-ATP and T_4 polynucleotide kinase. Cellulose acetate electrophoresis which is capable to distinguish the two nonamers was used for purity analysis of the labeled fragments.[16] Results indicated that the substition of the 4-triazolo group in the two nonamers proceeded to completion as expected. The labeled fragments were then digested by P_1-nuclease.[16] Their nucleotide sequences were confirmed by the 2-dimensional mobility-shift analysis of the nuclease digest[16] (Figure 5).

Table I Modification/phosphorylation of (2) and (11)

Nucleotide[a]/ Nucleoside[a]	Concentration (mole eq) Triazole	Tetrazole	ClØOPOCl$_2$[d]	Time	Product[c] (yield)
(2)	6.0		3.0	96 hr[b]	(3)(65%)
(2)		6.0	3.0	5 hr[b]	(12)(54%)
(2)		6.0	3.0	25 min[b]	(12)(15%),(11)(48%)
(2)	3.0		1.5	5 min[b]	(11)(81%)
(11)	3.5		1.7	96 hr	(3)(70%)
(11)		3.5	1.7	5 hr	(12)(63%)

[a] 0.2M solution in pyridine

[b] Time for the phosphodiester formation

[c] Product isolated after addition of β-cyanoethanol

[d] p-Chlorophenyl phosphodichloridate

EXPERIMENTAL

The fully protected mononucleotides (A,G,T) and 5'-O-dimethosytritylthymidine (2) were prepared according to published procedure.[11] p-Chlorophenyl phosphodichloridate (Alfa-Ventron), 1,2,4-triazole, 1,2,3,4-tetrazole (Aldrich), RP-2 silanized silcia gel 70-230 mesh (Brinkman), T_4-polynucleotide kinase, nuclease-P_1 enzyme (P-L Biochemical) and [γ-^{32}P]-ATP (New England Nuclear) were obtained commercially.

Uv absorption spectra were measured with Perkin-Elmer 402 and H'nmr spectra were obtained at 60 MHz with TMS as internal standard.

5'-O-Dimethoxytrityl-3'-O-(tert-butyldimethylsilyl)-thymidine (7)

5'-O-Dimethoxytritylthymidine (2) (20g, 36 mmol), imidazole (7g, 100 mmol), tert-butyldimethylchlorosilane (7g, 47 mmol) were stirred in pyridine (200 ml) for 3 hr. The mixture was diluted with water (200 ml) and extracted with dichloromethane (2 × 300 ml). The extract was washed with 2% sodium bicarbonate solution (200 ml) and the solvent was removed under vacuum. The residue was chromatographed on silanized silica gel column (45% water/acetone, v/v) to give (7) (21g, 89%) [Calcd. for $C_{37}H_{46}N_2O_7Si$: C,67.47; H,6.99; N,4.25. Found: C,67.33; H,7.09; N,4.27%], uv (MeOH) λ_{max} 268,

Table II Synthesis of the nonamer (4)

5'-Protected component[a] (mmol)	3'-Hydroxyl component[b] (mmol)	coupling[c] agent (mmol)	Time (min)	Product (% yield)
[(MeO)$_2$Tr]d$\underline{\overset{*}{H}}$-ClPh[d] (0.30)	dIsoG±CE (0.25)	0.50	15	[(MeO)$_2$Tr]d$\underline{\overset{*}{H}}$±IsoG ±CE (70)
[(MeO)$_2$Tr]d$\underline{\overset{*}{H}}$±IsoG -ClPh (0.15)	dbzC±bzA±bzA-OBz (0.12)	0.30	15	[(MeO)$_2$Tr]d$\underline{\overset{*}{H}}$±IsoG±bzC ±bzA±bzA-OBz (62)
[(MeO)$_2$Tr]dbzA±T± IsoG±IsoG-ClPh (0.08)	d$\underline{\overset{*}{H}}$±IsoG±bzC±bzA ±bzA-OBz (0.05)	0.16	15	[(MeO)$_2$Tr]dbzA±T±IsoG ±IsoG±$\underline{H}$±IsoG±bzC±bzA ±bzA-OBz (53)

[a]Abbreviations are as suggested by IUPAC-IUB, Biochemistry, 9, 4022 (1970) A phosphodiester linkage is representated by hyphen and phosphotriester linkage is represented by (±). Each internucleotidic phosphate is protected by p-chlorophenyl (ClPh) group.

[b]The 3'-hydroxyl group of the 3'terminal is protected in form of benzoate (OBz) or β-cyanoethyl p-chlorophenyl phosphotriester (±CE).

[c]Mesitylenesulfonyl-tetrazolide

[d]$\underline{\overset{*}{H}}$ is 5-methyl-4-triazolo-1-(β-D-2-deoxyribofuranosyl)-2(1H)-pyrimidinone

λ_{min} 254 nm. nmr ($CDCl_3$) δ7.53 (1H,s), 3.70 (6H,s), 1.42 (3H,s), 0.74 (9H,s).

4-(1,2,4-Triazolo)-1-(β-D-5-O-dimethoxytrityl-3-O-(tert-butyldimethylsilyl) -2-deoxyribofuranosyl)-5-methyl-2(1H)-pyrimidinone (8)

p-Chlorophenyl phosphodichloridate (10g, 40 mmol) was added to a solution of 5'-O-dimethoxytrityl-3'-(tert-butyldimethylsilyl)-thymidine (7) (15 g, 32 mmol) and 1,2,4-triazole (10g, 143 mmol) in pyridine (45 ml) at 4°. Then the mixture was stirred at room temperature for 4 days and was extracted with water (100 ml) and dichloromethane (200 ml). The organic extract was washed with 2% aqueous sodium bicarbonate and the solvent was removed in vacuo. The residue was chromatographed on short column of silanized silica

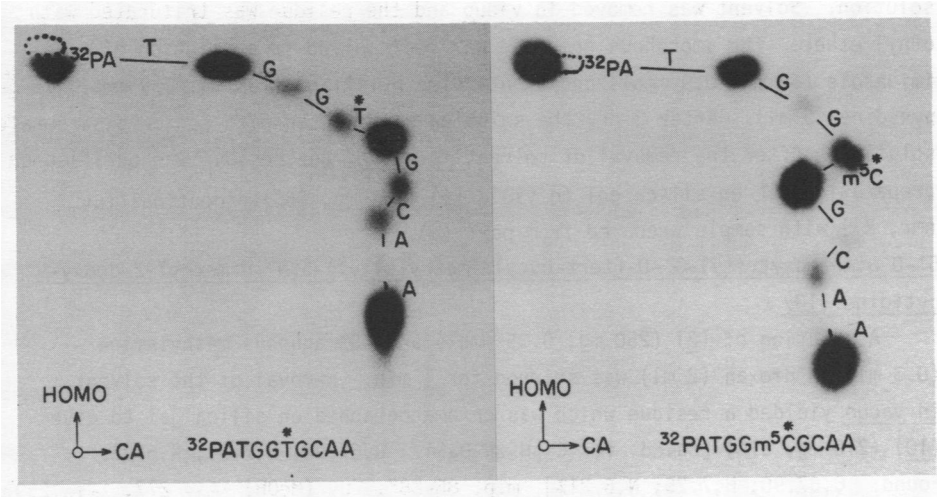

Fig. 5. Two-dimensional chromatographic fingerprints of the two nonanucleotides after partial digestion with nuclease-P_1. The first dimension is electrophoresis on cellulose acetate strip of PH 3.5 and the second dimension is homochromatography on 20 × 20 cm DEAE-cellulose plate in solvent Homo-V.

gel (43% water in acetone v/v) to yield (8) (12g, 73%). [Calcd. for $C_{39}H_{47}N_5O_6Si$: C,66.00; H, 6.62; N,9.87. Found: C,65.69; H,6.71; N,9.80%]. uv (MeOH) λ_{max} 327, λ_{min} 293 nm. nmr ($CDCl_3$) δ9.30 (1H,s), 8.43 (1H,s), 8.08 (1H,s), 3.82 (6H,s), 2.02 (3H,s), 0.90 (9H,s).

5'-O-Dimethoxytrityl-3'-O-(tert-butyldimethylsilyl)-5-methyl-2'-deoxycytidine (9)

a) A mixture of (8) (8.9g, 12.4 mmol), 20% aqueous ammonia (20 ml) and dioxan (100 ml) were stirred for 1 hour and was then diluted with dichloromethane (100 ml). The extract was washed with water (100 ml) and the solvent was removed in vacuo. The residue was purified by column chromatography on silica gel to give (9) (6.6g, 87%) [Calcd. for $C_{37}H_{47}N_3O_6Si$: C,67.57; H,7.15; N,6.37. Found: C,67.32; H,7.04; N,6.29%]. uv (MeOH) λ_{max} 278, λ_{min} 259 nm. nmr ($CDCl_3$) δ7.82 (1H,s), 3.88 (6H,s), 1.63 (3H,s), 0.95 (9H,s).

b) 5-Methyl-2'-deoxycytidine (100 mg, 0.41 mmol) and dimethoxytrityl chloride (152 mg, 0.45 mmol) were stirred in pyridine (5 ml) for 1 hr. The solution was diluted with water and was extracted with dichloromethane. The dichloromethane extract was washed with 2% aqueous sodium bicarbonate

solution. Solvent was removed in vacuo and the residue was triturated with ethyl ether. The amorphous compound was redissolved in a solution of imidazole (63 mg, 0.9 mmol) and t-butyldimethylsilane (135 mg, 0.9 mmol) in pyridine (5 ml). After 3 hr, the solution was diluted with sodium bicarbonate solution. After the removal of solvent in vacuo, the residue was purified on preparative tlc on silica gel to yield (9) (151 mg, 55%), identical (uv, nmr, R_f) with sample prepared from part (a).

5'-O-Dimethoxytrityl-3'-O-(tert-butyldimethylsilyl)-5,N^4-dimethyl-2-deoxycytidine (10)

A solution of (8) (250 mg, 0.35 mmol) and 40% aqueous methylamine (0.3 ml) in dioxan (2 ml) was stirred for 1 min. Removal of the solvent in vacuo yielded a residue which was chromatographed on silica gel to give (10) (210 mg, 91%) [Calcd. for $C_{38}H_{49}N_3O_6Si$: C,67.95; H,7.31; N,6.25. Found: C,67.90; H,7.25; N,6.21%] m.p. 86-88°. uv (MeOH) λ_{max} 277, λ_{min} 259 nm. nmr ($CDCl_3$) δ7.72 (1H,s), 3.82 (6H,s), 3.10 (3H,d,J=5Hz), 1.52 (3H,s), 0.87 (9H,s).

Regeneration of 5'-O-Dimethoxytrityl-3'-O-(tert-butyldimethylsilyl)-thymidine (7) from (8)

A solution of (8) (205 mg, 0.29 mmol), syn-4-nitrobenzaldoxime (100 mg, 0.6 mmol) and N^1,N^1,N^3,N^3-tetramethylguanidine (70 mg, 0.6 mmol) in dioxan (2 ml) was stirred for 5 hr. The mixture was then diluted with water (30 ml) and extracted with dichloromethane (2 × 50 ml). Removal of solvent from the organic extract yielded a residue which was purified by a short column of silanized silica gel to give (7) (163 mg, 85%) as a foam, identical with an authentic sample (H-nmr, uv, tlc).

5'-O-Dimethoxytritylthymidine 3'-p-chlorophenyl β-cyanoethyl phosphotriester (11)

5'-O-Dimethoxytritylthymidine (2) (4.0g, 7.2 mmol), triazole (1.6g, 22 mmol) and p-chlorophenyl phosphodichloridate (2.7g, 11 mmol) were stirred in pyridine (35 ml) for 5 min. Then β-cyanoethanol (10 ml) was added. After an hr, the mixture was diluted with water (100 ml) and extracted with dichloromethane (2 × 100 ml). The extract was washed with 2% aqueous sodium bicarbonate (100 ml) and the solvent was removed in vacuo. The residue was purified by chromatography on silanized silica gel to give (11) (4.5g, 81%), identical with a sample prepared by the established method.[11]

5-Methyl-4-triazolo-1-(β-D-5'-O-dimethoxytrityl-2'-deoxyribofuranosyl)-2(1H)-pyrimidinone 3'-p-chlorophenyl β-cyanoethyl phosphotriester (3)

a) 5'-O-Dimethoxytritylthymidine (2) (7.0g, 12 mmol), triazole (5.4g,

77 mmol) and p-chlorophenyl phosphodichloridate (9.4g, 38 mmol) were stirred in pyridine (60 ml) at 10° for 5 min, and then at room temperature for 96 hr. β-Cyanoethanol (20 ml) was added. After an hr, the mixture was diluted with water (200 ml) and extracted with dichloromethane (2 × 300 ml). The extract was washed with 2% aqueous sodium bicarbonate (100 ml) and the solvent was removed under vacuum. The residue was purified by chromatography on silanized silica gel to give (3) (6.9g, 65%) [Calcd. for $C_{42}H_{40}N_6O_9ClP$: C,60.21; H,4.77; N,10.03. Found: C,60.01; H,4.88; N,10.01%] uv (MeOH) λ_{max} 324, λ_{min} 290 nm. nmr ($CDCl_3$) δ9.32 (1H,s), 8.28 (1H,s), 8.11 (1H,s), 3.83 (6H,s), 2.74 (2H,q,J=5Hz), 2.07 (3H,s).

b) A mixture of (11) (1.0g, 1.2 mmol), triazole (0.28g, 4 mmol) and p-chlorophenyl phosphodichloridate (o.49g, 2 mmol) was stirred in pyridine (6 ml) at 10° for 5 min, and then at room temperature for 96 hr. The mixture was diluted with water (30 ml) and extracted with dichloromethane (2 × 50 ml). The extract was washed with 2% aqueous sodium bicarbonate (30 ml) and the solvent was removed in vacuo. The residue was chromatographed on silica gel to give (3) (0.66g, 70%), identical with the sample prepared in part (a).

5-Methyl-4-tetrazolo-1-(β-D-5'-O-dimethoxytrityl-2'-deoxyribofuranosyl)-2(1H)-pyrimidinone 3'-p-chlorophenyl β-cyanoethyl phosphotriester (12)

a) 5'-O-Dimethoxytritylthymidine (2) (1.0g,1.7 mmol), tetrazole (0.77g, 11 mmol) and p-chlorophenyl phosphodichloridate (1.3g, 5.5 mmol) were stirred in pyridine (10 ml) at 10° for 5 min, and then at room temperature for 5 hr. β-Cyanoethanol (3 ml) was added. After an hr, the mixture was diluted with water (30 ml) and was extracted with dichloromethane (2 × 50 ml). The extract was washed with 2% aqueous sodium bicarbonate (50 ml) and the solvent was removed in vacuo. The residue was chromatographed on silanized silica gel to give (12) (0.77g, 54%) [Calcd. for $C_{41}H_{39}N_7O_9ClP$: C,58.71; H,4.65; N,11.69. Found: C,58.38; H,4.62; N,11.60%]. uv (MeOH) λ_{max} 332, λ_{min} 291 nm. nmr ($CDCl_3$) δ9.70 (1H,s), 8.50 (1H,s), 3.82 (6H,s), 2.79 (2H,q,J=6Hz), 2.10 (3H,s).

b) A mixture of (11) (1.0g, 1.2 mmol), tetrazole (0.28g, 4 mmol) and p-chlorophenyl phosphodichloridate (0.49g, 2 mmol) was stirred in pyridine (6 ml) at 10° for 5 min, and then at room temperature for 5 hr. The mixture was diluted with water (2 ml) and extracted with dichloromethane (2 × 50 ml). The extract was washed with 2% aqueous sodium bicarbonate (30 ml) and the solvent was removed under vacuum. The residue was purified to give (12) (0.9g, 63%), identical with sample prepared in part (a).

General method for nucleotide condensation

This procedure is essentially similar to that previously reported for

the unmodified oligonucleotide.[11] As an example, the synthesis of [$(MeO)_2$Tr] d*H±IsoG±CE is described. The modified nucleotide H(3) (250 mg, 0.3 mmol) was dissolved in a solution of diisopropylamine (0.4 ml) and pyridine (2.5 ml). After 30 min, solvent was evaporated in vacuo to give the phosphodiester as foam, which was then washed with ethyl ether.

N-Benzoylguanosine 3'-β-cyanoethyl chlorophenyl phosphotriester, prepared from 5'-O-dimethoxytrityl-N-benzoylguanosine 3'-β-cyanoethyl chlorophenyl phosphotriester (210 mg, 0.25 mmol) via detritylation in 2% benzenesulfonic acid in methanol/dichloromethane (3:7 v/v), was added into a mixture of mesitylenesulfonyl tetrazolide (160 mg, 0.58 mmol) and the phosphodiester salt of (3) in pyridine (1 ml). After 15 min, the mixture was diluted with water (30 ml) and extracted with dichloromethane (2 × 40 ml). The extract was washed with 2% aqueous sodium bicarbonate (40 ml) and solvent was evaporated in vacuo. The residue was purified by chromatography on silica gel (40% acetone/dichloromethane) to yield the dimer [$(MeO)_2$Tr] d*H±IsoG±CE as foam.

The reaction conditions and other data of the synthesis of the nonamer are presented in Table II.

Detritylation of $(MeO)_2$TrATGG*HGCAA-OBz

The fully protected nonamer (30 mg) was treated with 2% benzenesulfonic acid in methanol/dichloromethane (3:7 v/v) (6 ml) for 5 min at 10°.[11] The solution was neutralized with 5% aqueous sodium bicarbonate (6 ml). The organic extract was washed with water and solvent was removed in vacuo to give the crude detritylated nonamer which, without further purification, were committed for final deprotection.

Final deprotection of the detritylated nonamer

a) Preparation of HO-$^{5'}$ATGGm^5CGCAA$^{3'}$-OH

5'-Detritylated nonamer (15 mg) was dissolved in a solution of dioxan (3 ml) and 20% aqueous ammonia (1 ml). After 3 hr, the solvent was evaporated in vacuo. The residue was dissolved in 20% aqueous ammonia/pyridine (1:2 v/v). After 48 hr at room temperature, the mixture was heated at 37° for 12 hr. Subsequent evaporation left a glassy residue which was purified by polyethyleneimine tlc. The purification and the isolation procedures have been reported previously.[11]

b) Preparation of HO-$^{5'}$ATGG*TGCAA$^{3'}$-OH

5'-Detritylated nonamer (15 mg, 0.005 mmol) was dissolved in a solution of syn-4-nitrobenzaldoxime (10 mg, 0.07 mmol) and N^1,N^1,N^3,N^3-tetramethylguanidine (7.7 mg, 0.07 mmol) in dioxan (1 ml). After 5 hr, dioxan was

evaporated in vacuo. The residue was redissolved in 20% aqueous ammonia/ pyridine (1:2 v/v). After 48 hr at room temperature, the mixture was heated at 37° for 12 hr. Subsequent work-up and purification by polyethyleneimine tlc were carried out according to published procedure.[11]

Nucleotidic sequence determination of the two nonamers

The nucleotidic sequences of the nonamers were determined according to well-established procedure (Figure 5).[16]

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