General Synthesis and Binding Affinity of Position-Selective Phosphonodiester- and Phosphotriester-Incorporated Oligodeoxyribonucleotides

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Synthesis of phosphonodiester- and phosphotriester-modified oligodeoxyribonucleotides has been accomplished via the phosphoramidite approach with allylic protection. The modification can be made at the selected position(s) of the oligomers. The efficiency of this method has been demonstrated by the synthesis of base-labile modified oligo(deoxyribonucleotide)s such as the methyl phosphates and phenylphosphonates. Melting temperatures of the duplexes containing these artificial strands indicate that the backbone-alternation, which is made at a single site, does not have a negative influence on the binding affinity to the complementary DNA.

Introduction

In recent years, given the increasing importance of antisense oligonucleotide chemotherapeutics, great efforts have been directed toward the invention of artificial oligo(deoxyribonucleotide)s with effective biological activities.[2] Oligo(deoxyribonucleotide)s whose internucleotidyl phosphodiester linkage is replaced by a nonionic function such as phosphonodiester or phosphotriester are a promising class of compounds, because (1) decrease of the negative charge on the phosphate oxygen atom may allow more efficient uptake into cellular membranes; (2) increase of lipophilicity and the removal of phosphate–phosphate electrostatic repulsion may induce strong hybridization with the target genes;[3] (3) the modification may bring about higher stability to nucleases. Since the antisense DNAs must have suitable water-solubility as well as lipophilicity, the analogs that receive the nonionic modification in the selected position(s) are particularly attractive. Furthermore, the altered oligomers serve as model and lead compounds for studying the duplex structure[4] and the reactivity of T4 kinase toward DNAs.[5] Certain analogs act as probes for elucidating specific interactions with proteins[6] and the complementary unmodified DNAs.[7] Accordingly, an efficient method for preparing the oligomers bearing a partially nonionic backbone has been strongly demanded, but it has not yet been viable. The major obstacle in the existing methods[4,8,9] is the harsh basic condition needed for the removal of the *N*-acyl protectors of the adenosine, cytidine, and guanosine moieties that cleaves the nonionic internucleotide linkage. Therefore only thymidylyl homologs requiring no *N*-protection have been prepared.[10] This paper describes a general way to synthesize such oligo(deoxyribonucleotide)s with a methyl- or phenylphosphonodiester backbone and alkyl, such as methyl, or phenyl phosphotriester internucleotide linkage. The efficiency is primarily based on the use of the allyloxycarbonyl (AOC) group as the protector for the nucleoside bases.[11]

Results and Discussion

The deoxyribonucleoside 3′-phenylphosphonamidite **4**[12] was prepared by the 1*H*-tetrazole/diethylamine-promoted condensation of the 3′-*O*-free nucleoside **1** and bis(diethylamino)phenylphosphine. The methyl, ethyl, and allyl deoxyribonucleoside 3′-phosphoramidites **5**–**7** were produced from **1** and the corresponding alkoxybis(diisopropylamino)phosphine.[11c] The reaction of **1** and $C_6H_5OPCl[N(i\text{-}C_3H_7)_2]$[13] gave the phenyl phosphoramidite analog **8**.

The synthesis of the phosphonodiester- and phosphotriester-modified DNA dimers in a solution phase was tried first in order to examine the efficiency of the allylic protection. Reaction of the thymidine 3′-phosphonamidite **4d** and 5′-*O*-free cytidine **2** in the presence of 1*H*-tetrazole followed by oxidation with *tert*-butyl hydroperoxide (TBHP)[14] and detritylation with dichloroacetic acid gave the AOC-protected dimer **9**. Removal of the AOC

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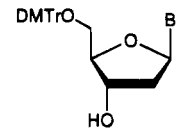
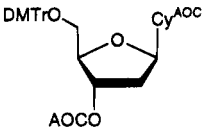


1a: B = Ad^{AOC}
1b: B = Cy^{AOC}
1c: B = Gu^{AOC}
1d: B = Th
1e: B = $Gu^{All, AOC}$



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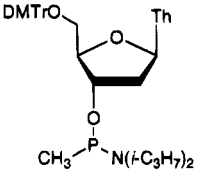

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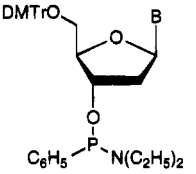
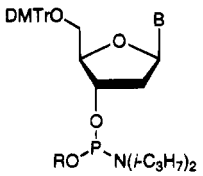
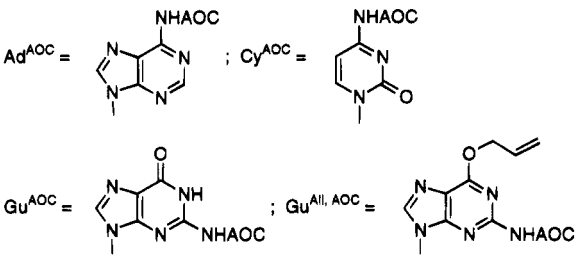


4a: B = Ad^{AOC}
4b: B = Cy^{AOC}
4d: B = Th
4e: B = $Gu^{All, AOC}$

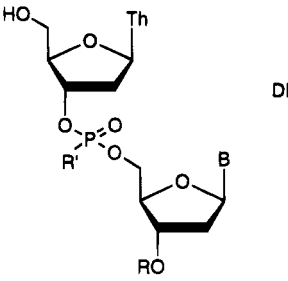


5: R = CH_3 a: B = Ad^{AOC}
6: R = C_2H_5 b: B = Cy^{AOC}
7: R = C_3H_5 c: B = Gu^{AOC}
8: R = C_6H_5 d: B = Th

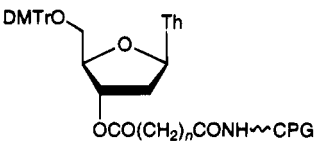


protectors using a $Pd[P(C_6H_5)_3]_4$-$P(C_6H_5)_3$ mixed catalyst system in the presence of diethylammonium hydrogen carbonate[15] afforded in a high overall yield the desired dinucleoside phenylphosphonodiester **10** as a ca. 1:1 mixture of the diastereomers. The HPLC of the product indicated the absence of the cleavage of the phenylphosphonate linkage. In a like manner, the methyl, ethyl, and phenyl phosphotriester analogs **11**–**13** were synthesized using the corresponding building blocks with AOC protectors.

The allyl protection method could also be applied to the solid-phase synthesis of the partially modified DNA oligomers. Synthesis of the monophenylphosphonodiester-incorporated pentamer, d[5′Tp(C_6H_5)CG-AT3′] (**14**), where p(C_6H_5) refers to phenylphosphonodiester linkage, is a typical example. The chain elongation was performed starting from the thymidine **15** covalently attached to a controlled pore glass (CPG) support via a long-(alkylamino)succinyl spacer arm. The reaction cycle consisting of (1) 1*H*-tetrazole-assisted condensation of the suitable phosphoramidite monomer **4d** (2.2 min) or **7a**–

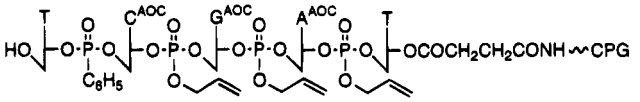


9: B = Cy^{AOC}; R = AOC; R′ = C_6H_5
10: B = Cy; R = H; R′ = C_6H_5
11: B = Cy; R = H; R′ = CH_3O
12: B = Cy; R = H; R′ = C_2H_5O
13; B = Cy; R = H; R′ = C_6H_5O



15: *n* = 2
16: *n* = 0

7c (1.1 min), (2) capping of the unreacted hydroxyl with acetic anhydride and 2,6-lutidine, (3) oxidation of the resulting phosphite with TBHP, and (4) detritylation with trichloroacetic acid gave the protected pentamer **17** in 96% overall coupling yield (trityl assay). The allyl and



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AOC protectors were then selectively removed by treatment with a mixture of tris(dibenzylideneacetone)dipalladium(0)-chloroform, triphenylphosphine, and diethylammonium formate in THF. Finally oligoDNA monophenylphosphonate **14** was detached from the solid support by exposure to concd ammonium hydroxide. The HPLC indicated that the product is a ca. 1:1 mixture of the diastereomers contaminated with few side products (Figure 1). The structure of **14** was confirmed by enzymatic digestion with snake venom phosphodiesterase and bacterial alkaline phosphatase giving a mixture of deoxyadenosine, deoxyguanosine, thymidine, and two diastereomers of Tp(C_6H_5)C in an expected ratio. The digestion product contained no *N*-allyloxycarbonylated nucleosides or allyl phosphates resulting from incomplete deprotection. This organometallic procedure was applicable to the synthesis of DNA methylphosphonates as well as phenyl or alkyl phosphotriester-incorporated DNA oligomers using commercially available **3** and the above-prepared **5**–**8** as monomer units. This procedure can be used to make not only monofunctionalized DNAs but multifunctionalized derivatives as well, as exemplified in Table 1. In order to prepare the methylated DNAs in high yield, a series of refinements were made. First, TBHP[14] is essential for the oxidation of the phosphite. When the oxidation was conducted by the conventional iodine-aqueous pyridine system,[16] the methyl ester suffered a nucleophilic attack of the iodide ion, resulting in the demethylated compound. Second, an excess amount of formic acid to butylamine is used in the removal of the allylic protectors in order to diminish the nucleophilicity of the amine. This is also necessary to avoid undesired demethylation via the S_N2 reaction between the methyl ester and the amine which is seen when an equimolar mixture of formic acid and butylamine is used as in the ordinary procedure.[11c] It is also important to

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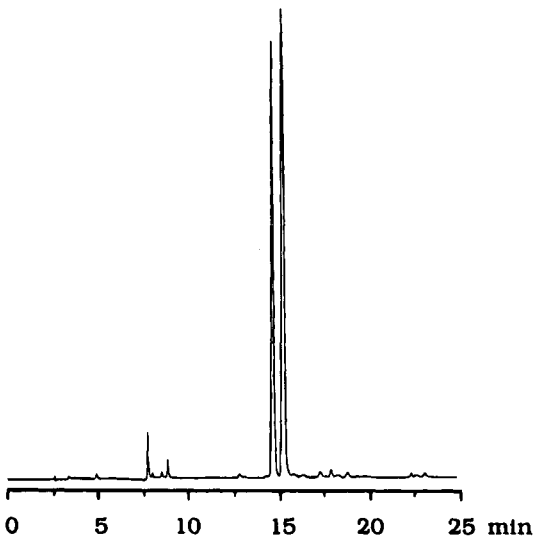

Figure 1. HPLC profile of crude d[5′Tp(C_6H_5)CGAT3′] (**14**).

Table 1. Synthesis of DNA Oligomers with Nonionic Internucleotide Linkage

DNA oligomer[a]	coupling yield, %[b,c] average	coupling yield, %[b,c] overall	purity, %[d]
d [5′Tp(CH_3)CGAT3′]	99.2	97	92
d[5′Tp(C_6H_5)CGAT3′] (**14**)	99.1	96	88
d[5′Tp(OCH_3)CGAT3′][e]	99.2	97	77
d[5′Tp(OC_2H_5)CGAT3′]	99.1	96	88
d[5′Tp(OC_6H_5)CGAT3′]	98.9	96	86
d[5′GACACp(C_6H_5)CCAAT3′] (**18**)	99.3	94	60
d[5′GACACp(OCH_3)CCAAT3′] (**19**)	99.4	95	55
d[5′GACACp(OC_2H_5)CCAAT3′] (**20**)	99.1	92	66
d[5′GACACp(OC_6H_5)CCAAT3′] (**21**)	99.1	92	79
d[5′Tp(C_6H_5)GTCGACACCCAATp-(C_6H_5)T3′]	97.2	72	76
d[5′Cp(OC_2H_5)GACACCCAATTCTGAAAAp-(OC_2H_5)T3′]	98.6	77	77
d[5′Tp(OC_6H_5)GTCGACACCCAATp-(OC_6H_5)T3′]	99.5	93	73

[a] A mixture of diastereomers. [b] Determined by the assay of DMTr cation. [c] Coupling yields for the *P*-modified amidites were generally >98%. [d] Content of the desired DNA oligomer in a crude product. Determined by HPLC analysis. [e] Synthesized using an oxalyl linker.

note that a long-(alkylamino)oxalyl chain, invented by Letsinger,[10] is used in place of the succinyl chain as a spacer linking the starting thymidine (see the compound **16**) to the solid support. This modification allows a mild release of the product from the linker, which minimizes the loss of the base-labile DNA methyl phosphotriester by hydrolysis. This approach completely eliminates *N*(3)-methylation of the thymine base[17] using a strong base[18] for deprotection and solid-phase removal of the DNA product which is often seen in the existing synthesis.

We attempted to prepare position-selectively modified DNA oligomers by the standard[19] or improved phosphoramidite method,[20] but obtained little, if any, desired products. For example, the monophenylphosphonate **14** was prepared in <10% yield by using acyl protectors, *i.e.*, benzoyl or isobutyryl, and 2-cyanoethyl, for the nucleoside base and internucleotide linkage, respectively, and hot concd ammonia for the deprotection.[19] Base-labile monophenylated DNA, d[5′Tp(OC_6H_5)CGAT3′], could not be obtained at all. The phenylphosphate was prepared in very low yield (35% purity) by the improved method using a 1:1 mixture of ethylenediamine and ethanol[20] for removal of the acyl protectors. These deprotection procedures failed to synthesize the monomethylated analog, d[5′Tp(OCH_3)CGAT3′].

Table 2. Tm's of the Duplexes formed from the Unmodified or Modified Decamer and the Complementary DNA

DNA oligomer	T_m/°C[a] polar isomer	T_m/°C[a] less polar isomer
d[5′GACACCCAAT3′] (**23**)	39.5	
d[5′GACACp(C_6H_5)CCAAT3′] (**18**)	35.0	37.5
d[5′GACACp(OCH_3)CCAAT3′] (**19**)	38.0[b]	40.5[c]
d[5′GACACp(OC_2H_5)CCAAT3′] (**20**)	38.0[b]	40.5[c]
d[5′GACACp(OC_6H_5)CCAAT3′] (**21**)	36.5	39.0

[a] The value was measured using 1 μM **18**–**21**, **23**, 1 μM **22**, 10 mM sodium phosphate buffer (pH 7.0), and 150 mM NaCl. [b] R_p isomer. [c] S_p isomer.

Diastereomers of d[5′GACACp(R)CCAAT3′] [R = C_6H_5 (**18**), CH_3O (**19**), C_2H_5O (**20**), and C_6H_5O (**21**)] were separated by HPLC. The absolute configuration of the stereogenic phosphorus center of the methyl and ethyl phosphotriester derivatives was determined on the basis of the Stec-Zon method.[9,21] Thus, the less polar isomer with large retention volume in the reversed-phase HPLC appears to have *S* configuration, and the polar isomer has *R* configuration. The stereochemistry of the phosphorus center in the phenylphosphono diester and the phenylphosphotriester were not be determined by extension of this method.[21] The ability of the diastereomeric analogs to hybridize with the complementary DNA, d[5′ATTGGGTGTC3′] (**22**), was investigated by comparison of the melting temperature (T_m) of the resulting duplex. As listed in Table 2, the diastereomer formed from the less polar isomer generally has a slightly higher Tm than the stereoisomer obtained from the polar isomer.[22] Figure 2 illustrates melting curves of the duplexes formed from the diasteromeric phenylphosphono derivative **18** and **22**, as well as the standard duplex made from d[5′GACACCCAAT3′] (**23**) and **22**. Thus the

(17) Nonoccurrence of the *N*-methylation was confirmed after enzymatic degradation of the modified oligodeoxyribonucleotide. For instance, the digestion of d[5′Tp(OCH_3)CGAT3′] with snake venom phosphodiesterase and bacterial alkaline phosphatase gave a mixture of deoxyadenosine, deoxyguanosine, thymidine, and Tp(OCH_3)C in an expected ratio and the HPLC showed no detectable peak due to *N*-methylthymidine.

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(21) Koziolkiewicz, M.; Uznanski, B.; Stec, W. J. *Nucleosides Nucleotides* **1989**, *8*, 185. For determination of the absolute configuration of the stereogenic phosphorus center of the phenylphosphonate **18** and the phenyl phosphate **21**, see footnote 22.

(22) The stability of a duplex containing a phosphonodiester- or phosphotriester-incorporated DNA is affected by the phosphorus configuration. In general, the derivative with an alkyl or alkoxy group turning to the major groove forms a less stable, lower melting duplex than the isomer with the substituent directed toward the outside of the groove. This empirical rule suggests that the stereogenic phosphorus atom of polar and less polar **18** has *S* and *R* configurations, respectively. In a similar way, the less and more polar isomers of **21** were tentatively assigned as the *S* and *R* compounds, respectively. See: refs 2 and (a) Summers, M. F.; Powell, C.; Egan, W.; Byrd, R. A.; Wilson, W. D.; Zon, G. *Nucleic Acids Res.* **1986**, *14*, 7421. (b) LaPlanche, L. A.; James, T. L.; Powell, C.; Wilson, W. D.; Uznanski, B.; Stec, W. J.; Summers, M. F.; Zon, G. *Ibid.* **1986**, *14*, 9081. (c) Pramanik, P.; Kan, L. *Biochemistry* **1987**, *26*, 3807. (d) Bower, M.; Summers, M. F.; Powell, C.; Shinozuka, K.; Regan, J. B.; Zon, G.; Wilson, W. D. *Nucleic Acids Res.* **1987**, *15*, 4915.

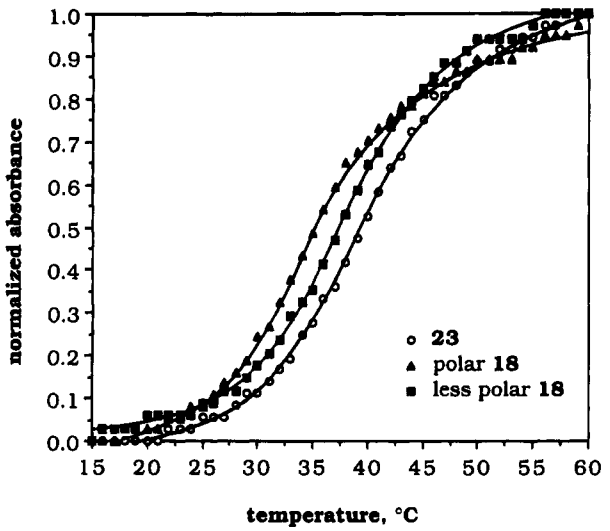

Figure 2. Dissociation of the duplexes formed from d-[5'GACACp(C_6H_5)CCAAT3'] (**18**) or d[5'GACACCCAAT3'] (**23**) and the complementary DNA **22**.

configuration of the incorporated nonionic ester moiety does affect the ability to hybridize with the complementary DNA, but the T_m values are close to the 39.5 °C seen with the standard duplex. The results show that the natural DNA **23** and artificial analogs **18**–**21**, either *R*- or *S*-configurated, have comparable affinities to the same complementary DNA **22**.

Conclusion

We realized a convenient, general synthesis of DNAs with mono- or multiphosphonodiester and -phosphotriester function(s) *at the selected position(s)*. The present synthetic method allows for the synthesis of oligo(deoxyribonucleotide)s bearing partially phenylphosphonodiester, methyl phosphotriester, or phenyl phosphotriester internucleotide linkage which are labile to bases such as ammonia and hydroxides. The success relies on the use of allylic protectors removable by a mild, nonbasic palladium-catalyzed reaction.[11] This protection in combination with the oxalyl linker[10] is particularly effective for the solid-phase synthesis of partially methylated DNAs. The T_m's of the duplexes containing the artificial DNAs indicate that the modification, which is made at single position, does not seriously affect the binding affinity to the complementary oligomer.[23] Thus, the analogs are expected to act as antisense molecules.

Experimental Section

General Methods and Materials. IR spectra were measured in KBr, unless otherwise noted. UV spectra were obtained in MeOH. NMR spectra were recorded in $CDCl_3$, unless otherwise stated. The ^1H chemical shifts are described as δ values in ppm relative to TMS. Chemical shifts quoted in ^{31}P NMR are downfield from 85% H_3PO_4. Elemental analysis was achieved at the Faculty of Agriculture, Nagoya University. HPLC was carried out using an ODS-5 μm column. 1*H*-Tetrazole was purified by sublimation in vacuo (0.1 mmHg) at 110 °C. N^6-[(Allyloxy)carbonyl]-5'-*O*-(*p*,*p*'-dimethoxytrityl)-2'-deoxyadenosine (**1a**),[11c] N^4-[(allyloxy)carbonyl]-5'-*O*-(*p*,*p*'-dimethoxytrityl)-2'-deoxycytidine (**1b**),[11c] N^2-[(allyloxy)carbonyl]-5'-*O*-(*p*,*p*'-dimethoxytrityl)-2'-deoxyguanosine (**1c**),[11c] 5'-*O*-(*p*,*p*'-dimethoxytrityl)thymidine (**1d**),[24] O^6-allyl-N^2-[(allyloxy)carbonyl]-5'-*O*-(*p*,*p*'-dimethoxytrityl)-2'-deoxyguanosine (**1e**),[15] 5'-*O*-(*p*,*p*'-dimethoxytrityl)thymidine 3'-(*N*,*N*-diisopropyl)phosphoramidites **5d**[25] and **6d**,[9b] 5'-*O*-(*tert*-butyldimethylsilyl)-2'-deoxycytidine,[26] and $Pd_2(dba)_3$–$CHCl_3$[27] were prepared by literature methods. The reagents for the solid-phase synthesis of DNAs by the Köster method was employed as supplied from Applied Biosystems, Inc (ABI). Acetonitrile solutions of nucleoside phosphoramidites (ca. 0.1 M) and 1*H*-tetrazole (0.5 M), a 1:1:8 Ac_2O–2,6-lutidine–THF mixture, and a 16% *N*-methylimidazole solution in THF were prepared immediately before use. The solid-phase synthesis of oligonucleotides was conducted on controlled pore glass (CPG) beads (pore size 500 Å) binding 1 μmol of a nucleoside. The condensation yields were monitored by quantitation of the released DMTr cation by the UV spectra in 60% $HClO_4$-EtOH (3:2) at 499 nm. The purity of the modified DNA oligomers was analyzed by HPLC with the following eluents and the gradient: solvent A = 5% CH_3CN in triethylammonium acetate (TEAA) buffer; solvent B = 20 or 30% CH_3CN in TEAA buffer/gradient = 0–2 min (A:B = 10:0), 2–3 min (A:B = 10:0–8:2), 3–33 min (A:B = 8:2–4:6), and 33–42 min (A:B = 4:6–0:10). Separation of the diastereomers of backbone-modified DNA oligomers was carried out by HPLC.

Bis(*N*,*N*-diethylamino)phenylphosphine. To a solution of $PhPCl_2$ (25 mL, 33.0 g, 0.18 mol) in THF (250 mL) under argon was slowly added Et_2NH (80 mL, 56.6 g, 0.77 mol) at room temperature and the resulting suspension was stirred for 1 h. Then the mixture was refluxed with vigorous stirring overnight. The resulting salts were removed by filtration under argon atmosphere and washed with THF. The combined filtrate and washings were concentrated. The residual material was distilled under reduced pressure to afford the title compound (41.3 g, 0.16 mol, 89%) as a colorless oil: bp 137–142 °C (0.1 mmHg); IR (neat) 1460, 1327, 1292, 1186 cm^{-1}; UV 245 nm (ϵ 7100); ^1H NMR 1.11 (t, 12H, J = 6.9 Hz), 3.02–3.16 (m, 8H), 7.16–7.48 (m, 5H); ^{31}P NMR 97.7. Anal. Calcd for $C_{14}H_{25}N_2P$: C, 66.62; H, 10.00; N, 11.10. Found: C, 66.51; H, 10.07; N, 11.39.

***O*-Ethyl *N*,*N*,*N*',*N*'-Tetraisopropylphosphorodiamidite.** To a stirred solution of *i*-Pr_2NH (147 mL, 106 g, 1.05 mol) in ether (400 mL) was added a solution of $EtOPCl_2$ (38.5 g, 263 mmol) in ether (50 mL) over 1 h at 0 °C. The resulting salts were removed by filtration, and the filtrate was concentrated. The residue was distilled in vacuo to afford the title compound (32.9 g, 0.119 mol, 45%) as a colorless oil; bp 67–68 °C (0.09 mmHg); IR (neat) 2973, 2488, 1462, 1362, 1250, 1186 cm^{-1}; ^1H NMR 1.10–1.35 (m, 27H), 3.40–3.69 (m, 6H); ^{31}P NMR 126.3. Anal. Calcd for $C_{14}H_{33}N_2OP$: C, 60.82; H, 12.06; N, 10.14. Found: C, 60.71; H, 12.37; N, 9.82.

***N*⁴,3'-*O*-Bis[(allyloxy)carbonyl]-2'-deoxycytidine (2).** To a stirred suspension of 1*H*-tetrazole (2.35 g, 33.5 mmol) and Et_3N (4.5 mL, 3.27 g, 32.3 mmol) in THF (50 mL) was added dropwise a solution of AOCCl (3.5 mL, 3.98 g, 33.0 mmol) in THF (30 mL) at 0 °C. The mixture was stirred for 30 min at the same temperature. The resulting salt was removed by filtration and washed well with THF. The combined filtrate and washing were concentrated under reduced pressure to give a viscous oil. The product was dissolved in THF (50 mL) and then mixed with a solution of 5'-*O*-(*tert*-butyldimethylsilyl)-2'-deoxycytidine (3.02 g, 8.85 mmol) in DMF (30 mL) and THF (100 mL). The mixture was refluxed overnight. After cooling to room temperature, the whole mixture was poured into water (300 mL) and extracted with a 1:1 mixture of EtOAc and hexane (200 mL × 3). The combined organic extracts were dried and concentrated. The residue was dissolved in THF (20 mL), and to this solution was added 1.0 M solution of tetrabutylammonium fluoride in THF (15 mL, 15 mmol). The

(23) The configuration of the stereogenic phosphorus atoms in fully methylated DNA oligomers is known to have little affect on the binding affinity.[8a]

(24) Schaller, H.; Weimann, G.; Lerch, B.; Khorana, H. G. *J. Am. Chem. Soc.* **1963**, *85*, 3821.
(25) Adams, S. P.; Kavka, K. S.; Wykes, E. J.; Holder, S. B.; Galluppi, G. R. *J. Am. Chem. Soc.* **1983**, *105*, 661.
(26) Ogilvie, K. K. *Can. J. Chem.* **1973**, *51*, 3799.
(27) Ukai, T.; Kawazura, H.; Ishii, Y. *J. Organomet. Chem.* **1974**, *65*, 253, and references cited therein.

mixture was stirred for 1 h at room temperature. Concentration gave a semisolid, which was recrystallized from EtOAc to afford **2** (2.91 g, 7.36 mmol, 83%) as colorless needles: mp 158–162 °C; IR 3306, 1748, 1651, 1609, 1503 cm^{-1}; UV 240 (ϵ 15 400), 293 nm (7900); ^1H NMR 2.46–2.75 (m, 2H), 3.12 (br s, 1H), 3.85–4.06 (m, 2H), 4.26–4.32 (m, 1H), 4.61–4.77 (m, 4H), 5.26–5.47 (m, 5H), 5.80–6.05 (m, 2H), 6.13–6.25 (m, 1H), 7.28 (d, 1H, J = 7.3 Hz), 7.70 (br s, 1H), 8.16 (d, 1H, J = 7.3 Hz). Anal. Calcd for $C_{17}H_{21}N_3O_8$: C, 51.64; H, 5.36; N, 10.63. Found: C, 51.60; H, 5.31; N, 10.41.

Synthesis of 2′-Deoxyribonucleoside 3′-Phosphonamidites or 3′-Phosphoramidites 4–6. Typical Procedure for the Preparation of N^6-[(Allyloxy)carbonyl]-5′-*O*-(p,p′-dimethoxytrityl)-2′-deoxyadenosine 3′-(*N*,*N*-Diethylphenylphosphonamidite) (4a). To a stirred solution of **1a** (2.20 g, 3.44 mmol) in CH_3CN (20 mL) under argon were added Et_2NH (127 mg, 1.74 mmol) and 1*H*-tetrazole (123 mg, 1.76 mmol). After 10 min, $PhP(NEt_2)_2$ (1.04 g, 4.12 mmol) was added and the mixture was stirred at room temperature overnight. 1*H*-Tetrazole (178 mg, 2.54 mmol) and $PhP(NEt_2)_2$ (573 mg, 2.27 mmol) were renewedly added and stirring was continued for additional 4 h. The mixture was poured into CH_2Cl_2 (100 mL) and washed with an aqueous $NaHCO_3$ solution (50 mL × 2). The organic layer was dried and concentrated to give a residual oil, which was subjected to silica gel (70 g) column chromatography with a 1:1–5:1 mixture of EtOAc and hexane containing a trace of Et_2NH to afford, after concentration, **4a** (1.31 g, 48% yield) as a ca. 1:1 diastereomeric mixture: IR 1759, 1611 cm^{-1}; UV 237 (ϵ 28 900), 267 nm (22 200); ^1H NMR 0.96 and 1.05 (t, 6H, J = 7.3 Hz), 2.64–3.12 (m, 6H), 3.35–3.51 (m, 2H), 3.75 and 3.78 (2 s, 6H), 4.37–4.47 (m, 1H), 4.77 (d, 2H, J = 5.9 Hz), 4.80–4.94 (m, 1H), 5.29 (d, 1H, J = 10.2 Hz), 5.41 (d, 1H, J = 17.2 Hz), 5.91–6.02 (m, 1H), 6.51–6.61 (m, 1H), 6.73–6.84 (m, 4H), 7.16–7.56 (m, 14H), 8.16 and 8.20 (2 s, 1H), 8.34 (br s, 1H), 8.70 and 8.72 (2 s, 1H); ^{31}P NMR 132.0, 132.7. Anal. Calcd for $C_{45}H_{49}N_6O_7P$: C, 66.15 H, 6.06; N, 10.29. Found: C, 66.14; H, 5.97; N, 10.39.

N^4-[(Allyloxy)carbonyl]-5′-*O*-(p,p′-dimethoxytrityl)-2′-deoxycytidine 3′-(*N*,*N*-diethylphenylphosphonamidite) (4b): yield 66%; IR 1748, 1672, 1626, 1557 cm^{-1}; UV 236 (ϵ 40 000), 284 nm (9700); ^1H NMR 0.92 and 1.04 (2 t, 6H, J = 6.9 Hz, 2.26–2.44 (m, 1H, H-2′), 2.69–3.10 (m, 5H), 3.40–3.59 (m, 2H), 3.75 and 3.80 (2 s each, 6H), 4.24–4.36 (m, 1H), 4.62–4.85 (m, 3H), 5.26–5.41 (m, 2H), 5.87–6.01 (m, 1H), 6.23 and 6.37 (2 t, 1H, J = 5.9 Hz), 6.75–7.00 (m, 5H), 7.19–7.50 (m, 14H), 8.25 and 8.39 (2 d, 1H, J = 7.3 Hz); ^{31}P NMR 133.6, 133.6. Anal. Calcd for $C_{44}H_{49}N_4O_8P$: C, 66.64; H, 6.24; N, 7.07. Found: C, 66.60; H, 6.24; N, 7.04.

5′-*O*-(p,p′-Dimethoxytrityl)thymidine 3′-(*N*,*N*-diethylphenylphosphonamidite) (4d): yield 79%; IR 1692, 1609, 1510 cm^{-1}; UV 235 (ϵ 29 800), 265 nm [15 500 (sh)]; ^1H NMR 0.91 and 1.04 (2 t, 6H, J = 6.9 Hz), 1.44 and 1.46 (2 s, 3H), 2.31–2.44 (m, 1H), 2.49–2.67 (m, 1H), 2.82–3.11 (m, 4H), 3.34–3.57 (m, 2H), 3.76, 3.77 and 3.79 (2 s, 6H), 4.22–4.31 (m, 1H), 4.72–4.84 (m, 1H), 6.42–6.54 (m, 1H), 6.77–6.88 (m, 4H), 7.21–7.52 (m, 14H), 7.65 and 7.68 (2 d, 1H, J = 1.0 Hz); ^{31}P NMR 132.5, 132.9. Anal. Calcd for $C_{41}H_{46}N_3O_7P$: C, 68.02; H, 6.42; N, 5.81. Found: C, 68.01; H, 6.49; N, 5.84.

O^6-Allyl-N^2-[(allyloxy)carbonyl]-5′-*O*-(p,p′-dimethoxytrityl)-2′-deoxyguanosine 3′-(*N*,*N*-diethylphenylphosphonamidite) (4e): yield 64%; IR 1759, 1609, 1510 cm^{-1}; UV 236 (ϵ 49 800), 270 nm [28 800 (sh)]; ^1H NMR 0.95 and 1.04 (2 t, 6H, J = 7.3 Hz), 2.58–3.14 (m, 6H), 3.34–3.53 (m, 2H), 3.74 and 3.76 (2 s, 6H), 3.32–3.41 (m, 1H, H-4′), 4.69 (d, 2H, J = 4.6 Hz), 4.81–4.91 (m, 1H), 5.08 (d, 2H, J = 5.0 Hz), 5.22–5.52 (m, 4H), 5.89–6.06 (m, 1H), 6.07–6.23 (m, 1H), 6.43–6.52 (m, 1H), 6.72–6.83 (m, 4H), 7.15–7.56 (m, 14H), 7.98 and 8.00 (1 s each, 1H); ^{31}P NMR 131.9, 132.6. Anal. Calcd for $C_{48}H_{53}N_6O_8P$: C, 66.03; H, 6.13; N, 9.63. Found: C, 65.99; H, 6.15; N, 9.56.

N^6-[(Allyloxy)carbonyl]-5′-*O*-(p,p′-dimethoxytrityl)-2′-deoxyadenosine 3′-(methyl *N*,*N*-diisopropylphosphoramidite) (5a): yield 90%; IR 1761, 1613, 1510 cm^{-1}; UV 236 (ϵ 29 300), 267 nm (sh); ^1H NMR 1.11–1.27 (m, 12H), 2.58–2.76 (m, 1H), 2.81–2.95 (m, 1H), 3.28–3.70 (m, 7H), 3.77 (s, 6H), 4.29–4.38 (m, 1H), 4.70–4.84 (m, 3H), 5.29 (dd, 1H, J = 1.3 and 10.6 Hz), 5.41 (dd, 1H, J = 1.3 and 17.2 Hz), 5.99 (ddt, 1H, J = 10.6, 17.2, and 5.6 Hz), 6.44–6.53 (m, 1H), 6.74–6.84 (m, 4H), 7.15–7.43 (m, 9H), 8.05–8.16 (m, 2H), 8.68 (s, 1H); ^{31}P NMR 149.6, 149.8. Anal. Calcd for $C_{42}H_{51}N_6O_8P$: C, 63.13; H, 6.45; N, 10.52. Found: C, 63.10; H, 6.26; N, 10.66.

N^4-[(Allyloxy)carbonyl]-5′-*O*-(p,p′-dimethoxytrityl)-2′-deoxycytidine 3′-(methyl *N*,*N*-diisopropylphosphoramidite) (5b): yield 85%; IR 1748, 1672, 1557 cm^{-1}; UV 236 (ϵ 31 900), 284 nm (8800); ^1H NMR 1.02–1.28 (m, 12H), 2.15–2.37 (m, 1H), 2.64–2.83 (m, 1H), 3.22–3.64 (m, 7H), 3.80 (s, 6H), 4.17–4.23 (m, 1H), 4.52–4.78 (m, 3H), 5.29 (dd, 1H, J = 1.3 and 10.6 Hz), 5.37 (dd, 1H, J = 1.3 and 17.3 Hz), 5.94 (ddt, 1H, J = 10.6, 17.3, and 5.6 Hz), 6.19–6.35 (m, 1H), 6.79–6.97 (m, 5H), 7.20–7.50 (m, 9H), 8.25 and 8.31 (d, 1H, J = 7.6 Hz); ^{31}P NMR 149.3 and 150.1. Anal. Calcd for $C_{41}H_{51}N_4O_9P$: C, 63.54; H, 6.65; N, 7.23. Found: C, 63.58; H, 6.53; N, 7.24.

N^2-[(Allyloxy)carbonyl]-5′-*O*-(p,p′-dimethoxytrityl)-2′-deoxyguanosine 3′-(methyl *N*,*N*-diisopropylphosphoramidite) (5c): yield 85%; IR 1703, 1610, 1510, cm^{-1}; UV 237 (ϵ 26 200), 250 (sh), 259 (sh), 275 nm (sh); ^1H NMR 1.00–1.36 (m, 12H), 2.44–2.65 (m, 1H), 2.69–2.87 (m, 1H), 3.17–3.88 (m, 13H), 4.19–4.37 (m, 1H), 4.56–4.81 (m, 3H), 5.31 (d, 1H, J = 10.6 Hz), 5.36 (d, 1H, J = 16.8 Hz), 5.91 (ddt, 1H, J = 10.6, 16.8, and 5.6 Hz), 6.15–6.34 (m, 1H), 6.66–6.92 (m, 4H), 7.15–7.50 (m, 9H), 7.77 and 7.79 (2 s, 1H); ^{31}P NMR 149.5, 149.6. Anal. Calcd for $C_{42}H_{51}N_6O_9P$: C, 61.90; H, 6.32; N, 10.32. Found: C, 61.88; H, 6.35; N, 10.16.

N^6-[(Allyloxy)carbonyl]-5′-*O*-(p,p′-dimethoxytrityl)-2′-deoxyadenosine 3′-(ethyl *N*,*N*-diisopropylphosphoramidite) (6a): yield 67%; IR 1761, 1611, 1510 cm^{-1}; UV 236 (ϵ 24 300), 267 nm (20 100); ^1H NMR 1.03–1.28 (m, 15H), 2.60–2.78 (m, 1H), 2.82–2.95 (m, 1H), 3.29–3.80 (m, 12H), 4.31–4.40 (m, 1H), 4.70–4.82 (m, 3H), 5.29 (dd, 1H, J = 1.3 and 10.2 Hz), 5.41 (dd, 1H, J = 1.3 and 17.2 Hz), 6.00 (ddt, 1H, J = 10.2, 17.2, and 5.9 Hz), 6.43–6.54 (m, 1H), 6.74–6.82 (m, 4H), 7.13–7.43 (m, 9H), 8.11–8.22 (m, 2H), 8.69 (s, 1H); ^{31}P NMR 146.9, 147.2. Anal. Calcd for $C_{43}H_{53}N_6O_8P$: C, 63.52; H, 6.58; N, 10.34. Found: C, 63.51; H, 6.30; N, 10.31.

N^4-[(Allyloxy)carbonyl]-5′-*O*-(p,p′-dimethoxytrityl)-2′-deoxycytidine 3′-(ethyl *N*,*N*-diisopropylphosphoramidite) (6b): yield 92%; IR 1748, 1672, 1557, 1508 cm^{-1}; UV 236 (ϵ 32 300), 284 nm (8900); ^1H NMR 1.11–1.38 (m, 15H), 2.21–2.38 (m, 1H), 2.63–2.87 (m, 1H), 3.34–3.88 (m, 12H), 4.17–4.28 (m, 1H), 4.50–4.76 (m, 3H), 5.29 (dd, 1H, J = 1.3 and 10.2 Hz), 5.36 (dd, 1H, J = 1.3 and 17.2 Hz), 5.94 (ddt, 1H, J = 10.2, 17.2, and 5.9 Hz), 6.26–6.34 (m, 1H), 6.77–6.94 (m, 5H), 7.20–7.41 (m, 9H), 8.24 and 8.30 (2 d, 1H, J = 7.3 Hz); ^{31}P NMR 147.0, 147.9. Anal. Calcd for $C_{42}H_{53}N_4O_9P$: C, 63.94; H, 6.77; N, 7.10. Found: C, 63.95; H, 6.72; N, 7.00.

N^2-[(Allyloxy)carbonyl]-5′-*O*-(p,p′-dimethoxytrityl)-2′-deoxyguanosine 3′-(ethyl *N*,*N*-diisopropylphosphoramidite) (6c): yield 75%; IR 1709, 1611 cm^{-1}; UV 237 (ϵ 26 100), 250 (sh), 259 (sh), 275 nm (sh); ^1H NMR 1.00–1.39 (m, 15H), 2.43–2.65 (m, 1H), 2.73–2.88 (m, 1H), 3.20–3.93 (m, 12H), 4.20–4.37 (m, 1H), 4.57–4.79 (m, 3H), 5.32 (d, 1H, J = 10.9 Hz), 5.35 (d, 1H, J = 17.2 Hz), 5.91 (ddt, 1H, J = 10.9, 17.2, and 5.6 Hz), 6.18–6.27 (m, 1H), 6.66–6.88 (m, 4H), 7.17–7.49 (m, 9H), 7.77 and 7.78 (2 s, 1H); ^{31}P NMR 146.9, 147.0. Anal. Calcd for $C_{43}H_{53}N_6O_9P$: C, 62.30; H, 6.46; N, 10.14. Found: C, 62.32; H, 6.29; N, 10.05.

Preparation of the Nucleoside 3′-(Phenyl *N*,*N*-Diisopropylphosphoramidite) 8. Typical Procedure for the Synthesis of N^6-[(Allyloxy)carbonyl]-5′-*O*-(p,p′-dimethoxytrityl)-2′-deoxyadenosine 3′-(Phenyl *N*,*N*-Diisopropylphosphoramidite) (8a). To a stirred solution of **1a** (1.01 g, 1.59 mmol) in CH_2Cl_2 (10 mL) were added *i*-Pr_2NEt (1.1 mL, 816 mg, 6.31 mmol) and $PhO(i\text{-}Pr_2N)PCl$ (826 mg, 3.18 mmol). The mixture was stirred for 1.5 h and was diluted with CH_2Cl_2 (100 mL). The organic solution was washed with brine (50 mL × 2), dried, and concentrated. The residue was subjected to silica gel column chromatography eluted with a 1:2–4:1 mixture of ether and hexane containing a trace of *i*-Pr_2NH. Concentration of the eluents afforded a gummy material, which was triturated from pentane at −78 °C to afford **8a** (925

mg, 68% yield) as a ca 1:1 mixture of diastereomers: IR 1761, 1611, 1510 cm^{-1}; UV 237 (sh), 267 nm (ϵ 26 200); ^1H NMR 1.03–1.28 (m, 12H), 2.59–2.76 (m, 1H), 2.83–2.98 (m, 1H), 3.29–3.50 (m, 2H), 3.62–3.80 (m, 8H), 4.33–4.42 (m, 1H), 4.75 (d, 2H, J = 5.6 Hz), 4.80–4.92 (m, 1H), 5.27 (dd, 1H, J = 1.3 and 10.2 Hz), 5.38 (dd, 1H, J = 1.3 and 17.2 Hz), 6.00 (ddt, 1H, J = 10.2, 17.2, and 5.6 Hz), 6.40–6.54 (m, 1H), 6.70–6.80 (m, 4H), 6.92–7.06 (m, 5H), 7.12–7.41 (m, 9H), 8.11 and 8.16 (2 s, 1H), 8.69 and 8.70 (2 s, 1H); ^{31}P NMR 147.0 and 147.3. Anal. Calcd for $C_{47}H_{53}N_6O_8P$: C, 65.56; H, 6.22; N, 9.76. Found: C, 65.62; H, 6.02; N, 9.67.

N^4-[(Allyloxy)carbonyl]-5′-*O*-(*p*,*p*′-dimethoxytrityl)-2′-deoxycytidine 3′-(phenyl *N*,*N*-diisopropylphosphoramidite) (8b): yield 80%; IR 1745, 1672, 1508 cm^{-1}; UV 235 (ϵ 32 700), 284 nm (8900); ^1H NMR 1.00–1.30 (m, 12H), 2.17–2.41 (m, 1H), 2.63–2.91 (m, 1H), 3.32–3.96 (m, 10H), 4.15–4.34 (m, 1H), 4.52–4.84 (m, 3H), 5.26 (d, 1H, J = 10.2 Hz), 5.34 (d, 1H, J = 17.5 Hz), 5.92 (ddt, 1H, J = 10.2, 17.5, and 5.6 Hz), 6.17–6.38 (m, 1H), 6.74–7.05 (m, 10H), 7.15–7.44 (m, 9H), 8.24 (d, 1H, J = 6.5 Hz); ^{31}P NMR 147.0, 147.4. Anal. Calcd for $C_{46}H_{53}N_4O_9P$: C, 66.02; H, 6.38; N, 6.69. Found: C, 66.13; H, 6.42; N, 6.59.

N^2-[(Allyloxy)carbonyl]-5′-*O*-(*p*,*p*′-dimethoxytrityl)-2′-deoxyguanosine 3′-(phenyl *N*,*N*-diisopropylphosphoramidite) (8c): yield 53%; IR 1707, 1610 cm^{-1}; UV 237 (ϵ 26 400), 250 (sh), 260 (sh), 276 (sh), 284 nm (8900); ^1H NMR 1.00–1.38 (m, 12H), 2.46–2.59 (m, 1H), 2.71–2.92 (m, 1H), 3.22–3.40 (m, 2H), 3.60–3.83 (m, 8H), 4.27–4.38 (m, 1H), 4.60–4.84 (m, 3H), 5.26–5.42 (m, 2H), 5.82–6.00 (m, 1H), 6.14–6.27 (m, 1H), 6.68–6.81 (m, 4H), 6.88–7.04 (m, 5H), 7.09–7.44 (m, 9H), 7.73 and 7.77 (2 s, 1H); ^{31}P NMR 146.9, 147.0. Anal. Calcd for $C_{47}H_{53}N_6O_9P$: C, 64.36; H, 6.10; N, 9.58. Found: C, 64.30; H, 6.16; N, 9.46.

5′-*O*-(*p*,*p*′-Dimethoxytrityl)thymidine 3′-(phenyl *N*,*N*-diisopropylphosphoramidite) (8d): yield 88%; IR 1693, 1609, 1510 cm^{-1}; UV 237 (sh), 268 nm (ϵ 12 000); ^1H NMR 1.07–1.26 (m, 12H), 1.44 (s, 3H), 2.22–2.42 (m, 1H), 2.49–2.61 (m, 1H), 3.28–3.40 (m, 1H), 3.43–3.57 (m, 1H), 3.60–3.87 (m, 8H), 4.15–4.27 (m, 1H), 4.68–4.84 (m, 1H), 6.36–6.51 (m, 1H), 6.71–7.06 (m, 9H), 7.13–7.48 (m, 9H), 7.59 and 7.64 (s, 1H); ^{31}P NMR 146.9, 147.6. Anal. Calcd for $C_{43}H_{50}N_3O_8P$: C, 67.26; H, 6.56; N, 5.47. Found: C, 67.31; H, 6.54; N, 5.46.

Synthesis of Authentic Samples of the Nucleotide Dimers with the Modified Backbone, 10–13. Typical Procedure for the Preparation of Thymidylyl-(3′)-2′-deoxycytidyl Phenylphosphonate (10). To a solution of the 3′-amidite **4d** (357 mg, 0.49 mmol) and 5′-*O*-unprotected nucleoside **2** (130 mg, 0.33 mmol) in CH_3CN (5 mL) was added 1*H*-tetrazole (3.17 mmol) and the resulting mixture stirred at room temperature for 4 h. A 1.0 M solution of TBHP in toluene (1.0 mL, 1.0 mmol) was added and stirring was continued for additional 30 min. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with an aqueous solution saturated with $NaHCO_3$ (50 mL × 2) followed by brine (50 mL × 2). The organic solution was dried and concentrated. The residue was dissolved in CH_2Cl_2 (30 mL). After the addition of $CHCl_2COOH$ (0.6 mL, 938 mg, 7.27 mmol), the solution was stirred at room temperature for 20 min. The reaction was quenched by the addition of a saturated solution of $NaHCO_3$ (50 mL). The mixture was extracted with CH_2Cl_2 (20 mL × 3). The organic solution was dried and concentrated. The resulting residual material was chromatographed on a short silica gel column with a 50:1–10:1 mixture of CH_2Cl_2 and MeOH to afford the AOC protected nucleotide dimer (180 mg). To a stirred suspension of the dimer (147 mg, 0.19 mmol) and diethylammonium hydrogen carbonate (291 mg, 2.15 mmol) in CH_2Cl_2 (10 mL) was added a solution of $Pd[PPh_3]_4$ (23.2 mg, 20.1 μmol) and PPh_3 (7.8 mg, 29.7 μmol) in CH_2Cl_2 (1.0 mL), and the mixture was stirred at room temperature for 30 min. Concentration gave a viscous oil, which was purified by preparative TLC with a 4:1 mixture of CH_2Cl_2 and MeOH to afford **10** (111 mg, 71% overall yield) as a ca 1:1 diastereomeric mixture: IR 3428, 1655 cm^{-1}; UV 266 nm (ϵ 15 900); ^1H NMR (CD_3OD) 1.87 (s, 3H), 2.01–2.62 (m, 4H), 3.57–3.80 (m, 2H), 4.08–4.11 (m, 1H), 4.24–4.41 (m, 4H), 5.12–5.20 (m, 1H), 5.78–5.84 (m, 1H), 6.19–6.36 (m, 2H), 7.56–7.87 (m, 7H); ^{31}P NMR (CD_3OD) 18.9, 19.3. MS (FAB) m/z 592 (M^+ + 1), 614 (M^+ + Na).

Thymidylyl-(3′)-2′-deoxycytidine *O*-methyl ester (11): overall yield 58%; IR 3217, 1694, 1549 cm^{-1}; UV 272 nm (ϵ 15 800); ^1H NMR (CD_3OD) 1.89 (s, 3H), 2.10–2.24 (m, 1H), 2.29–2.58 (m, 3H), 3.75–3.88 (m, 5H), 4.04–4.12 (m), 4.19–4.43 (m, 4H), 5.10–5.14 (m, 1H), 5.94 and 5.94 (2 d, 1H, J = 7.6 Hz), 6.22–6.41 (m, 2H), 7.69–7.88 (m, 2H); ^{31}P NMR (CD_3OD) −0.5. MS (FAB) m/z 546 (M^+ + 1), 568 (M^+ + Na).

Thymidylyl-(3′)-2′-deoxycytidine *O*-ethyl ester (12): overall yield 54%; IR 3350, 1651 cm^{-1}; UV 267 nm (ϵ 15 500), ^1H NMR (CD_3OD) 1.33–1.42 (m, 3H), 1.88 (s, 3H), 2.10–2.23 (m, 1H), 2.28–2.59 (m, 3H), 3.75–3.88 (m, 2H), 4.04–4.12 (m, 1H), 4.13–4.43 (m, 6H), 5.10–5.14 (m, 1H), 5.94 and 5.95 (2 d, 1H, J = 7.6 Hz), 6.22–6.36 (m), 7.73–7.83 (m, 2H); ^{31}P NMR (CD_3OD) −1.67, −1.69. MS (FAB) m/z 560 (M^+ + 1), 582 (M^+ + Na).

Thymidylyl-(3′)-2′-deoxycytidine *O*-phenyl ester (13): overall yield 49%; IR 3391, 1659 cm^{-1}; UV 267 nm (ϵ 15 600); ^1H NMR (CD_3OD) 1.88 (s, 3H), 1.99–2.18 (m, 1H), 2.29–2.60 (m, 3H), 3.64–3.85 (m, 2H), 4.03–4.53 (m, 5H), 5.15–5.27 (m, 1H), 5.84 and 5.86 (2 d, 1H, J = 7.6 Hz), 6.18–6.37 (m, 2H), 7.18–7.47 (m, 5H), 7.65 and 7.70 (2 d, 1H, J = 7.6 Hz), 7.74–7.81 (m, 1H); ^{31}P NMR (CD_3OD) −7.1, −7.0; MS (FAB) m/z 608 (M^+ + 1), 630 (M^+ + Na).

Typical Procedure for Deprotection and CPG-Detachment of DNA Oligomers Partially Bearing Nonionic Internucleotide Linkage. (a) Synthesis Using a Succinyl Linker. The CPG beads binding the protected dGACACp-(C_6H_5)CCAAT (ca. 1 μmol) were washed with THF (1.0 mL) and dried in vacuo. A freshly prepared homogeneous solution of the Pd catalyst by heating a mixture of $Pd_2(dba)_3$–$CHCl_3$ (44.3 mg, 42.8 μmol), Ph_3P (108 mg, 0.412 mmol), Et_2NH (0.23 mL, 0.16 g, 2.2 mmol), and HCOOH (89 mL, 0.11 g, 2.4 mmol) in THF (1.8 mL) was added to the CPG supports charged in a serum-capped test tube, and the heterogeneous mixture was shaken with a Voltex mixer. The mixture was heated at 50 °C for 1 h occasionally with shaking as above. The supernatant fluid was separated by decantation and the CPG supports were washed by the reported method.[11c] The cleaned CPG supports were treated with concentrated ammonia (1.5 mL) at 25 °C for 0.5 h in the synthesis with a succinyl linker to afford 80.0 OD_{260} of d[5′GACACp(C_6H_5)CCAAT3′] (**18a**).

(b) Synthesis Using an Oxalyl Linker. After deprotection, the CPG supports were treated with 5% NH_4OH/CH_3OH solution (1.5 mL) at 25 °C for 5 min. The supernatant was concentrated (ca. 15 mmHg) to give the DNA oligomer.

Measurement of Melting Temperatures. A solution of the modified DNA, the complementary DNA, sodium phosphate buffer (pH 7.0), and NaCl, where the concentration of each substance is 1 μM, 1 μM, 10 mM, and 150 mM, respectively, was prepared. The UV spectra of the solution were measured at 260 nm from 15–60 °C at ramp of 1 °C/5 min to determine the melting temperatures.

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Supplementary Material Available: ^1H NMR, ^{31}P NMR, and ^{31}P-^1H 2D-NMR spectra for compounds **10–13** (12 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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