The Allylic Protection Method in Solid-Phase Oligonucleotide Synthesis. An Efficient Preparation of Solid-Anchored DNA Oligomers

Yoshihiro Hayakawa,*[1a] Shigeharu Wakabayashi,[1a] Hisatoyo Kato,[1b] and Ryoji Noyori*[1b]

Contribution from the Chemical Instrument Center and Department of Chemistry, Nagoya University, Chikusa, Nagoya 464-01, Japan. *Received June 27, 1989*

Abstract: The first efficient synthesis of solid-anchored DNA oligomers has been realized; it relies on the use of allyl and (allyloxy)carbonyl groups as protectors of internucleotide linkage and nucleoside bases, respectively, in conjunction with palladium chemistry. The preparation is performed via a phosphoramidite approach on controlled pore glass supports with a long-chain alkylamine spacer. The fully protected DNA is deblocked on the solid supports by removal of the allylic protecting groups by treatment with a mixture of the tris(dibenzylideneacetone)dipalladium(0)–chloroform complex, triphenylphosphine, butylamine, and formic acid at 50 °C for 0.5–1 h. Exposure of the solid-bound materials to concentrated ammonia at room temperature for 2 h affords free DNAs of excellent purity. The efficiency of this method has been demonstrated by the synthesis of d(5'CAAGTTGATGAACAATACTTCATACCTAAACT3') (32mer), d(5'TATCGGACACGTAACCCTCCCATGTC-GATGCAAATCTTTAACA3') (43mer), and d(5'TATGGGCCTTTTGATAGGATGCTCACCGAGCAAAACCAAG-AACAACCAGGAGATTTTATT3') (60mer).

Chemical synthesis of oligodeoxyribonucleotides on solid supports is acquiring a greater importance in response to recent impressive advances in molecular biology, particularly in recombinant DNA research and technology. The purpose of the present study is 2-fold. The first goal is synthesis of DNA oligomers that remain linked to the solid supports.[2] Preparation of solid-anchored DNAs finds unlimited applications in biochemical, biomedical, and recombinant DNA research. Such DNAs can be used for affinity chromatography[3] for rapid, reliable analysis and isolation of specific complementary DNA (cDNA) and RNA sequences,[4] enrichment of desired genes in a cDNA library, solid-phase amplification of DNA,[5] purification of DNA-binding proteins,[6] diagnosis of infections and genetic diseases, etc. The second aim is to develop a new method for synthesis of highly pure DNA oligomers. Although a variety of synthetic approaches along this line have been developed,[7] the purity of the synthesized DNA is often variable, depending on the sequence and length, and is not generally satisfactory. We recently reported that allyl and (allyloxy)carbonyl (AOC) groups serve as efficient protectors for internucleotide bonds and nucleoside bases, respectively, in solution-phase synthesis of nucleic acids.[8] These allylic protectors can be removed by a palladium(0)-catalyzed reaction under mild conditions, resulting in DNA oligomers in high yield. This paper describes a successful application of this organometallic methodology to automated solid-phase DNA synthesis based on the phosphoramidite approach.[9] The conventional spacers linking the nucleotides and solid matrices are stable to the nonbasic deblocking conditions, which allow ready access to DNAs free of protecting groups but still bound to the solid supports.

Results and Discussion

We selected as targets three oligomers having different lengths and sequences: a 32mer, d(5'CAAGTTGATGAACAATAC-TTCATACCTAAACT3') (**1**), a 43mer, d(5'TATCGGACACGT-AACCCTCCCATGTCGATGCAAATCTTTAACA3') (**2**), and a 60mer, d(5'TATGGGCCTTTTGATAGGATGCTCACC-GAGCAAAACCAAGAACAACCAGGAGATTTTATT3') (**3**). Oligomers **1** and **3** are known as parts of a DNA sequence of yolk sac tumor proteoglycan cDNA pPG1,[10] and **2** is a sequence involved in *Brevibacterium lactofermentum* plasmid pAM330 DNA.[11]

Synthesis of Allyl- and AOC-Protected 2'-Deoxyribonucleoside 3'-Phosphoramidites. The requisite 3'-O-free, *N*-AOC-protected nucleoside units were prepared from commercially available compounds. The conditions suitable for introducing the AOC group to heteroaromatic moieties appeared to be highly dependent on the nucleoside structures. Conversion of 2'-deoxyadenosine to the adenosine derivative **7** was achieved via a four-step sequence: (1) 4-(dimethylamino)pyridine- (DMAP-) catalyzed dimethoxytritylation, forming **4**,[12] (2) standard silylation with *tert*-butyldimethylsilyl chloride and imidazole, giving **5**, (3) allyloxycarbonylation with 1-[(allyloxy)carbonyl]tetrazole in hot THF, affording **6**, and (4) desilylation using tetrabutylammonium fluoride (TBAF). In the case of 2'-deoxycytidine, the sugar

(1) (a) Chemical Instrument Center. (b) Department of Chemistry.

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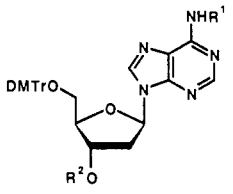
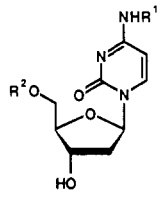
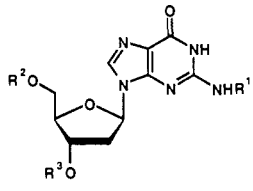
hydroxyls were first silylated with trimethylsilyl chloride[12] and then the AOC protector was introduced to the nucleoside base



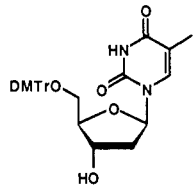
4, $R^1 = R^2 = H$
5, $R^1 = H$; R^2 = TBDMS
6, R^1 = AOC; R^2 = TBDMS
7, R^1 = AOC; $R^2 = H$



8, R^1 = AOC; $R^2 = H$
9, R^1 = AOC; R^2 = DMTr



10, $R^1 = H$; $R^2 = R^3$ = TBDMS
11, R^1 = AOC; $R^2 = R^3$ = TBDMS
12, R^1 = AOC; $R^2 = R^3 = H$
13, R^1 = AOC; R^2 = DMTr; $R^3 = H$



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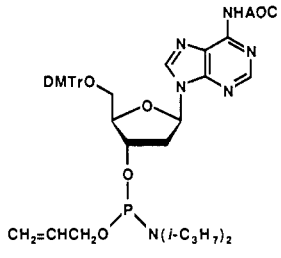
with allyl 1-benzotriazolyl carbonate. Subsequent removal of the silyl protectors with saturated aqueous sodium hydrogen carbonate, providing the *N*-AOC derivative **8**, followed by dimethoxytritylation, furnished the desired 3′-O-free compound **9**. The guanosine derivative **13** was prepared from the 3′,5′-di-O-silylated compound **10**[13] by (1) allyloxycarbonylation with a combination of *tert*-butylmagnesium chloride and allyl chlorocarbonate, forming **11**, (2) desilylation with TBAF, giving **12**, and (3) dimethoxytritylation. The thymidine derivative **14** is commercially available.[14]

These 3′-O-free intermediates were then converted to the nucleoside 3′-phosphoramidite monomer units by condensation with (allyloxy)bis(diisopropylamino)phosphine, assisted by 1*H*-tetrazole and diisopropylamine.[15] The isolated yields of **15**, **16**, **17**, and **18**, after trituration with cold pentane, were 91, 88, 85, and 100%,



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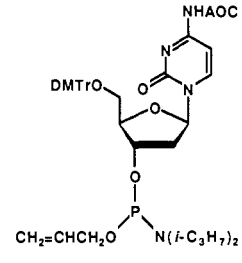

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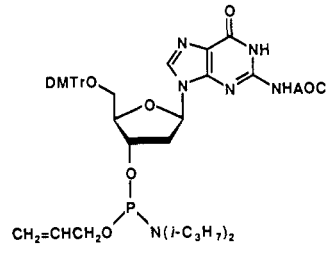

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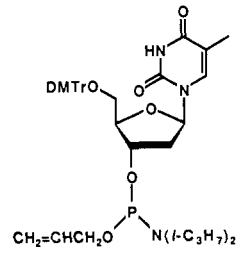

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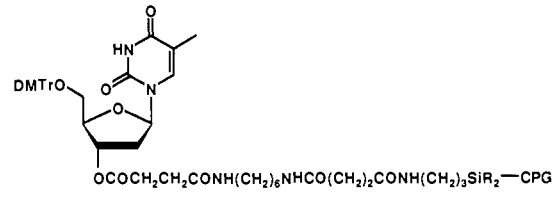
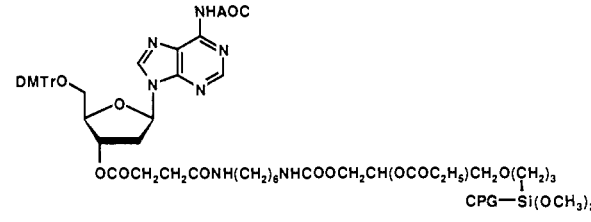
respectively. The amidite structures were fully supported by elemental analysis and spectral data, including the ^{31}P NMR signals appearing at δ 148–149 ppm. These solid amidite monomers were stable and showed no deterioration after storage for 1 year at −30 °C.

Synthesis of Solid-Anchored Oligodeoxyribonucleotides. The chain elongation was achieved on a 1-μmol scale on an Applied Biosystems Model 381A DNA synthesizer, starting from thymidine (**19**) or *N*-AOC-adenosine (**20**) covalently attached at the



19, B = Th
21, B = Ad^{Bz}



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3′-hydroxyl to controlled pore glass (CPG) supports (500-Å pore size) via a long-chain alkylamine spacer arm.[16] Table I outlines the synthetic cycle, requiring 10.8 min, where the phosphite intermediates were oxidized by anhydrous *tert*-butyl hydroperoxide[17] instead of the conventional aqueous iodine–pyridine mixture. After the final condensation, deprotection of the oligomers was conducted by the following sequence: First, the DMTr protector of the 5′-hydroxyl terminus was removed by treatment with trichloroacetic acid to give CPG-supported oligoDNAs in which all NH_2 moieties of dA, dC, and dG units were blocked by single AOC groups and the internucleotide bonds were protected by allyl groups. Then the products were exposed to a mixture of the tris(dibenzylideneacetone)dipalladium(0)–chloroform complex $[Pd_2(dba)_3–CHCl_3]$ (2.5 equiv/allyl), triphenylphosphine (25 equiv/allyl), and a large excess of butylamine and formic acid in THF at 50 °C for 0.5–1 h, which deblocked the two kinds of allylic protectors all at once.[8] The CPG-supported material was finally washed with a sodium *N*,*N*-diethyldithiocarbamate (ddtc) aqueous solution of pH 9.7 to ensure removal of any contaminated palladium residue.[18] Thus we were able to realize a first efficient synthesis of solid-bound oligoDNAs free of protective groups.

Preparation of Oligonucleotides of High Purity. Treatment of the solid-anchored products with concentrated ammonia at ambient temperature for 2 h effected hydrolysis of the succinate linkage to detach DNA chains from the support. HPLC analysis of the products obtained by digestion of the oligomer by snake venom phosphodiesterase and bacterial alkaline phosphatase[19] confirmed that there was full removal of the protective groups

(13) Ogilvie, K. K. *Can. J. Chem.* **1973**, *51*, 3799.
(14) This compound is also available according to the literature: Schaller, H.; Weimann, G.; Lerch, B.; Khorana, H. G. *J. Am. Chem. Soc.* **1963**, *85*, 3821.
(15) Barone, A. D.; Tang, J.-Y.; Caruthers, M. H. *Nucleic Acids Res.* **1984**, *12*, 4051. See also: Hamamoto, S.; Takaku, H. *Chem. Lett.* **1986**, 1401.
(16) Efcavitch, J. W.; McBride, L. J.; Eadie, J. S. In *Biophosphates and Their Analogues*; Bruzik, K. S., Stec, W. J., Eds.; Bioactive Molecules Series 3; Elsevier: Amsterdam, 1987; pp 65–70.
(17) Hayakawa, Y.; Uchiyama, M.; Noyori, R. *Tetrahedron Lett.* **1986**, *27*, 4191.
(18) Atomic absorption spectroscopy of the 32mer, for example, indicated that the product contained ca. 1 Pd atom/DNA chain without ddtc treatment. Washing with ddtc followed by purification by PAGE and ethanol precipitation diminished the contamination to ca. 0.003 Pd atom/DNA chain. Although the ddtc solution is slightly basic (pH 9.7), no detachment of DNA chains took place under these washing conditions.
(19) Eadie, J. S.; McBride, L. J.; Efcavitch, J. W.; Hoff, L. B.; Cathcart, R. *Anal. Biochem.* **1987**, *165*, 442.

Table I. Reaction Sequence of the Solid-Phase Synthesis

step	operation	reagent	time, min
1	washing	CH_3CN	0.5
2	detritylation	3% Cl_3CCOOH–CH_2Cl_2	1.8
3	washing	CH_3CN	3.0
4	coupling	0.1 M phosphoramidite–CH_3CN + 0.5 M (4-nitrophenyl)tetrazole–CH_3CN–THF	1.1
5	washing	CH_3CN	0.2
6	capping	Ac_2O–2,6-lutidine–THF (1:1:8) + 6.5% DMAP–THF	0.4
7	oxidation	1.1 M *t*-C_4H_9OOH–CH_2Cl_2	0.8
8	washing	CH_3CN	0.6

Table II. Synthesis of DNA Oligomers

oligoDNA	method[a]	coupling yield, %[b] average	coupling yield, %[b] overall	purity, %[c]	isolated, yield, %[d]
32mer **1**	A	99.3	81	93	60
32mer **1**	B	98.8	69	47	22
43mer **2**	A	99.2	73	65	17
43mer **2**	B	98.3	49	9	3
60mer **3**	A	99.3	66	70	25
60mer **3**	B	98.7	47	20	6

[a] A: allyl–AOC method. B: current method using the sequence of Table I. [b] Determined by the assay of DMTr cation. [c] Content of the desired oligoDNA in a whole nucleotide mixture determined by bio-image analysis. [d] Isolated by preparative electrophoresis on polyacrylamide gel.

and no nucleoside modification.[20] HPLC of the digests obtained from the 60mer **3** showed only four kinds of the parent nucleosides, and no *N*-AOC-protected compounds were detected. The experimentally derived base composition, dA:dC:dG:T = 20.5:12.5:12.6:15.0, agreed well with the calculated ratio, 20:12:13:15. Furthermore, the digested product was inert to 5′-^{32}P labeling with adenosine 5′-[γ-^{32}P]triphosphate using T4 polynucleotide kinase. This fact showed the absence of oligomers longer than a dimer, ensuring the complete elimination of the allyl protectors for phosphate linkages.

Reference samples of the three oligoDNAs, **1**–**3**, were prepared from **19** or **21**, on the same apparatus as in Table I,[21] with commercially supplied nucleoside phosphoramidites as monomer units with acyl (benzoyl for dA and dC and isobutyryl for dG) and 2-cyanoethyl for protection of the amino and phosphate functionalities, respectively.[9d] After detritylation, decyanoethylation from the phosphate linkage and detachment of the CPG-anchored product were effected under the standard conditions by treatment with concentrated ammonia at room temperature for 2 h. Heating with ammonia at 55 °C for 12 h caused deacylation, resulting in the final products.

The average coupling yield in the solid-phase synthesis was determined by the usual spectrophotometric quantitation of released *p,p′*-dimethoxytrityl cation. The yield per elongation cycle in the allyl–AOC method was 99.2–99.3%, attaining overall coupling yields of 81% for 32mer **1**, 73% for 43mer **2**, and 66% for 60mer **3**, respectively. Table II compares the results obtained by this and by the current amidite method using the commerical monomer units. The crude DNA oligomers were subjected to kination with T4 polynucleotide kinase in the presence of adenosine 5′-[γ-^{32}P]triphosphate to afford the ^{32}P-labeled 5′-monophosphates.

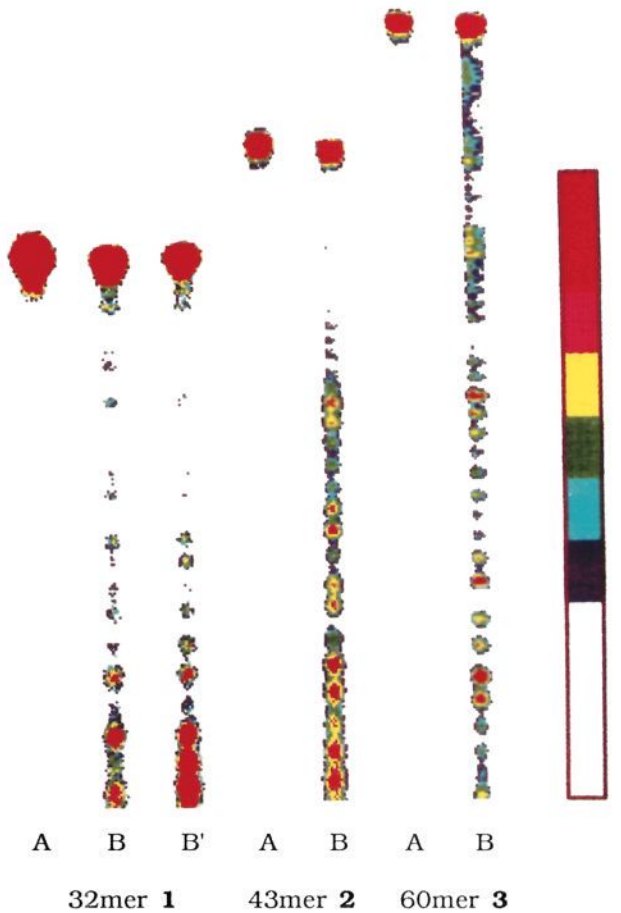

Figure 1. Bio-image chromatogram of the crude synthetic oligoDNAs. Lane A: allyl–AOC method. Lane B: current method using the sequence of Table I. Lane B′: current standard method. Color level indicator: red (higher intensity) to blue (lower intensity).

Figure 1 illustrates the bio-image chromatogram[22] of the resulting products. The oligonucleotides made by the allyl–AOC method give a chromatogram showing only feeble spots due to short-chain DNAs, which are not negligible with respect to the reference samples. The bio-image analysis estimated the 60mer contents of the crude products to be 70% (allyl–AOC) and 20% (conventional). The oligomer content by the allyl–AOC method is consistent with that expected from the coupling yield, indicating that the deprotection and polymer detachment are achievable in near-quantiative yield.[23] In contrast, the 60mer content obtained by the conventional method is considerably lower than that calculated from the coupling yield. Thus, during removal of *N*-benzoyl and *N*-isobutyryl protectors under harsh conditions,[24]

(20) HPLC analysis of the digests indicated the absence of undesired N(3)-allylation of thymidine. For N(3)-methylation in the synthesis using a methyl phosphoramidite unit, see: (a) Gao, X.; Gaffney, B. L.; Senior, M.; Riddle, R. R.; Jones, R. A. *Nucleic Acids Res.* **1985**, *13*, 573. (b) Urdea, M. S.; Ku, L.; Horn, T.; Gee, Y. G.; Warner, B. D. *Nucleic Acids Symp. Ser.* **1985**, *16*, 257.

(21) The standard procedure was slightly modified. Somewhat improved results were obtained by the use of (4-nitrophenyl)tetrazole in place of 1*H*-tetrazole for coupling (step 4) and *tert*-butyl hydroperoxide in place of aqueous iodine for oxidation (step 7). The bio-image chromatogram shown in Figure 1 illustrates the differences in purity of the 32mers obtained by the current method using the sequence of Table I (lane B) and the current standard method (land B′).

(22) The bio-image analyzer, based on imaging plate chromatography, recently developed at Fuji Photo Film Co., Ltd., Kanagawa, Japan, gives much better linear correlation between intensity of the spots on the chromatogram and amounts of labeled samples, compared with the usual autoradiographic or densitographic analysis. See: Amemiya, Y.; Miyahara, J. *Nature* **1988**, *336*, 89.

(23) Sequential treatments of the final polymer-bound product with (1) acetic anhydride–DMAP–2,6-lutidine (capping), (2) $Pd_2(dba)_3$–$CHCl_3$ and $P(C_6H_5)_3$ in the presence of *n*-$C_4H_9NH_2$ and HCOOH (removal of allylic protecting groups), and (3) concentrated NH_4OH at ambient temperature (deacetylation and detachment) gave an oligomer capable of undergoing T4 kinase phosphorylation with ATP. This fact confirms that the mild NH_4OH treatment can remove 5′-terminal acetyl groups from polymer-bound and unbound DNA products.

(24) Some recently invented *N*-acyl protectors such as phenoxyacetyl are removable under very mild conditions (aqueous NH_3, room temperature, 0.5–6 h) that allow synthesis of DNA oligomers of high purity and good yields: Schulhof, J. C.; Molko, D.; Teoule, R. *Tetrahedron Lett.* **1987**, *28*, 51. See also: Wu, T.; Ogilvie, K. K.; Pon, R. T. *Ibid.* **1988**, *29*, 4249.

ca. 60% of the product was lost, mainly by rupture of the internucleotide bonds. The difference in the synthetic efficiency was also shown by preparative electrophoresis of the crude products on polyacrylamide gels (PAGE), affording pure 60mer **3** in 25% (allyl–AOC) and 6% (conventional) yields. A similar trend was seen with 32mer **1**. The 43mer **2** is very sensitive, and the product could be obtained with only 9% purity by the conventional method. However, the purity of **2** prepared by the new procedure was >7-fold higher. Thus it has been revealed that (1) the efficiency of the coupling cycle of the present method is comparable with or slightly better than that of the current procedure,[25] (2) removal of allyl and 2-cyanoethyl protectors from the internucleotide bonds proceeds equally well under mild conditions, causing no problems, and (3) AOC is removable from the nucleoside base much more facilely than the acyl groups. Overall, the allyl–AOC procedure gives satisfactory synthetic efficiency and higher product purities than the conventional procedure, as shown by at least three typical experiments performed by us (Table II). Sufficient purity of the synthetic DNAs was also substantiated by a biological test. Thus, for instance, in a Tris–Mn^{2+} assay system with dGTP as a substrate,[26] the 43mer prepared by the allyl–AOC method showed the same primer activity for terminal deoxynucleotidyltransferase (EC 2.7.7.31) as that shown by the commercial $(dA)_{12-18}$.

Conclusion

The allyl–AOC procedure is characterized by the use of an organometallic methodology to remove all the protecting groups. The allylic groups serve as general protectors of NH_2 and phosphate moieties. It is worth noting that, in the 60mer synthesis, 104 allylic protective groups are removed from a CPG-supported oligomer in almost 100% overall yield by a single palladium-catalyzed reaction. This method is eminently practicable for isolating target DNAs without tedious, time-consuming chromatography, because all unbound contaminants, including unreacted reagents, can be removed by simple washing; the final, highly pure oligomers can be obtained by evaporation of an aqueous solution after detachment from the linker bound to the CPG support. *An even more significant feature is the capability of direct preparation of solid-anchored DNA oligomers.* Its applicability in molecular biology and diagnostics is enormous.

Experimental Section

General Methods and Materials. Melting points (mp) are uncorrected. Infrared (IR) spectra were measured with a JASCO IR-800 spectrometer. Ultraviolet (UV) spectra were obtained in methanol, unless otherwise noted, on a Hitachi 228 UV–visible light spectrometer. ^1H and ^{31}P nuclear magnetic resonance (NMR) spectra in $CDCl_3$ were recorded on a JEOL FX-90 or JEOL GX-500 instrument. The chemical shifts are described as δ values in ppm relative to a $Si(CH_3)_4$ standard for ^1H NMR. ^{31}P NMR chemical shifts quoted are downfield from 85% H_3PO_4. Elemental analysis was achieved at the Faculty of Agriculture, Nagoya University. High-performance liquid chromatography (HPLC) using a Nucleosil column (ODS-7 μm, 300 Å) or Wakosil column (ODS-5 μm, 100 Å) was carried out on a JASCO Trirotar-III chromatograph with a JASCO UVIDEC-100-III UV-absorption detector. Analytical thin-layer chromatography (TLC) was conducted on a plate coated with E. Merck Kieselgel 60 F_{254} (0.25-mm thickness). Most reactions were carried out in serum-capped, oven-dried, and argon-purged flasks. For enzymatic reactions, 1.5-mL Eppendorf tubes were used.

Acetonitrile was predried over activated 3A molecular sieves and distilled from CaH_2. THF was continuously refluxed from sodium benzophenone ketyl and distilled immediately before use. Dichloromethane for solid-phase DNA synthesis was distilled from CaH_2 with bubbling of argon gas. Pyridine, triethylamine, *N,N*-dimethylformamide (DMF), diisopropylamine, allyl chlorocarbonate (AOCCl), and butylamine were distilled from CaH_2. Doubly distilled water was employed for enzymatic reactions.

4-(Dimethylamino)pyridine (DMAP) and 1*H*-tetrazole were dried over P_2O_5 at 50 °C in vacuo overnight. 5′-*O*-(*p,p*′-Dimethoxytrityl)-2′-deoxyadenosine (**4**),[12] 3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (**10**),[13] 5′-*O*-(*p,p*′-dimethoxytrityl)thymidine (**14**),[14] and $Pd_2(dba)_3$–$CHCl_3$[27] were prepared according to literature methods. N^6-Benzoyl-5′-*O*-(*p,p*′-dimethoxytrityl)-2′-deoxyadenosine 3′-(2-cyanoethyl *N,N*-diisopropylphosphoramidite) (Lot No. A8L034), N^4-benzoyl-5′-*O*-(*p,p*′-dimethoxytrityl)-2′-deoxycytidine 3′-(2-cyanoethyl *N,N*-diisopropylphosphoramidite) (Lot No. A8K036), N^2-isobutyryl-5′-*O*-(*p,p*′-dimethoxytrityl)-2′-deoxyguanosine 3′-(2-cyanoethyl *N,N*-diisopropylphosphoramidite) (Lot No. A8K044), and 5′-*O*′(*p,p*′-dimethoxytrityl)-thymidine 3′-(2-cyanoethyl *N,N*-diisopropylphosphoramidite) (Lot No. A8L033) were commercially available from Applied Biosystems, Inc. Other commercially supplied nucleosides were used after drying by heating at 50–60 °C over P_2O_5 in vacuo or azeotropic removal of water with pyridine. Enzymes were obtained from Cooper Biomedical and Toyobo. Triethylammonium adenosine 5′-[γ-^{32}P]triphosphate salt was supplied from Amersham. All other organic substances, including solvents for extractive workup, chromatography, and recrystallization, were employed after simple distillation or recrystallization of the commercially supplied materials.

All solid-phase syntheses were carried out on a Model 381A DNA synthesizer of Applied Biosystems, Inc. The oligonucleotides were prepared according to the synthetic cycle (10.8 min/cycle) outlined in Table I in the text. Acetonitrile solutions (ca. 0.1 M) of nucleoside phosphoramidites, an acetonitrile–THF (1:1) mixture (0.5 M) of (4-nitrophenyl)tetrazole as an activator, an acetic anhydride–2,6-lutidine–THF (1:1:8) mixture, and a 6.5% DMAP–THF solution were freshly prepared prior to use. Dichloromethane solutions, including 3% Cl_3CCOOH and 1.1 M t-C_4H_9OOH, were stored in tightly sealed bottles under argon. Controlled pore glass (CPG) supports (pore size 500 Å) binding 1 μmol of a nucleoside were used. The condensation yields were monitored by quantitation of the released DMTr cation at 499 nm, which was collected on a Pharmacia Frac-100 fraction collector and dissolved in 60% $HClO_4$–EtOH (3:2) after concentration.

3′-*O*-(*tert*-Butyldimethylsilyl)-5′-*O*-(*p,p*′-dimethoxytrityl)-2′-deoxyadenosine (5). To a solution of 2′-deoxyadenosine (3.05 g, 12.1 mmol), Et_3N (2.50 mL, 17.9 mmol), and DMAP (80.5 mg, 0.659 mmol) in pyridine (100 mL) was added *p,p*′-dimethoxytrityl chloride (DMTrCl) (5.72 g, 16.9 mmol), and the mixture was stirred at 13 °C for 12 h in the dark. The reaction mixture was poured into water (650 mL) and extracted with ethyl acetate (150 mL, 100 mL × 2). The combined organic layer was concentrated to a gum, which was dried in vacuo for 5 h to afford 5′-*O*-(*p,p*′-dimethoxytrityl)-2′-deoxyadenosine (**4**).[12] Compound **4** was dissolved in DMF (50 mL), and to this solution were added imidazole (1.02 g, 15.0 mmol) and *tert*-butyldimethylsilyl chloride (2.65 g, 17.6 mmol). After the resulting mixture was stirred at 13 °C for 4.5 h, water was added (250 mL) and the mixture was extracted with a 1:1 mixture of hexane and ethyl acetate (100 mL × 2, 50 mL). The combined organic layer was washed with brine (50 mL), dried, and concentrated. The crude product was subjected to silica gel column chromatography [silica gel (150 g), CH_2Cl_2–MeOH (30:1)] to afford the crystalline product **5**: mp 151–152 °C (5.12 g, 63%); IR (KBr) 3330, 3160, 2960, 2940, 2860, 1670, 1650, 1510, 1260, 1180 cm^{-1}; UV λ_{max} 236 nm (ε 23 400), 260 (16 100); ^1H NMR 0.03, 0.33 [2 s's, 6 H, $Si(CH_3)_2$], 0.89 (s, 9 H, Si-*t*-C_4H_9), 2.01 (ddd, 1 H, J = 3.6, 6.0, and 13.2 Hz, H-2′), 2.81 (dt, 1 H, J = 6.0 and 13.2 Hz, H-2′), 3.36 (t, 2 H, J = 4.5 Hz, H-5′), 3.82 (s, 6 H, $2OCH_3$), 4.12 (dd, 1 H, J = 4.5 and 7.5 Hz, H-4′), 4.63 (m, 1 H, H-3′), 5.83 (br, 2 H, NH_2), 6.46 (t, 1 H, J = 6.0 Hz, H-1′), 6.73–6.95 (m, 4 H, protons ortho to OCH_3 of DMTr), 7.20–7.53 (m, 9 H, aromatic protons of DMTr), 8.06 (s, 1 H, H-2), 8.35 (s, 1 H, H-8). Anal. ($C_{37}H_{45}N_5O_5Si$) C, H, N.

THF Solution of 1-[(Allyloxy)carbonyl]tetrazole (AOCTet). AOCCl (12.0 mL, 0.113 mol) was added to a mixture of 1*H*-tetrazole (7.0 g, 0.100 mol) and Et_3N (15.0 mL, 0.108 mol) in THF (150 mL) at 0 °C over 15 min. The mixture was stirred for 30 min. The resulting precipitates were filtered off through a pad of Celite 545 and washed with THF. The combined THF solution including AOCTet was concentrated to ca. one-tenth volume.

N^6-[(Allyloxy)carbonyl]-5′-*O*-(*p,p*′-dimethoxytrityl)-2′-deoxyadenosine (7). A solution of **5** (18.0 g, 0.027 mol) and AOCTet (ca. 0.1 mol) in THF (150 mL) was heated to 70 °C for 3 h. After the mixture was cooled to 25 °C, concentration under a reduced pressure gave a viscous oil, which was diluted with ethyl acetate and poured into saturated aqueous $NaHCO_3$. After the mixture was extracted with ethyl acetate (300 mL), the combined organic extracts were washed with brine (50 mL) and dried over $MgSO_4$. Concentration under a reduced pressure

(25) For DNA synthesis in the <100mer range using an Applied Biosystems Model 381A synthesizer, the coupling is considered to proceed in 99% average yield (Appl. *Biosystems DNA Synth. Notes* **1986**, June 4). Another literature reports 99.5–99.8% yield per cycle on a Biosearch Model 8600 system (Giles, J. W. *Am. Biotechnol. Lab.* **1985**, Nov/Dec).

(26) Kaneda, T.; Kuroda, S.; Koiwai, O.; Yoshida, S. *J. Biochem.* (*Tokyo*) **1981**, *90*, 1421.

(27) Ukai, T.; Kawazura, H.; Ishii, Y. *J. Organomet. Chem.* **1974**, *65*, 253. This compound is commercially available from Aldrich.

gave residue **6**, which was dissolved in THF (100 mL) and treated with 1.0 M tetrabutylammonium fluoride (TBAF) in THF (50 mL, 0.05 mol) at 25 °C for 12 h. After removal of the solvent, the resulting material was dissolved in ethyl acetate (150 mL), washed with brine, and dried over $MgSO_4$. The dried solution was concentrated, and the residue was subjected to chromatography on silica gel (300 g) and eluted with a mixture of $CHCl_3$–MeOH–Et_3N (50:1:trace to 30:1:trace) to afford **7**: mp 84–86 °C (14.4 g, 84%); IR (KBr) 3410, 2960, 2940, 1760, 1620, 1515, 1470, 1255, 1220, 1180 cm^{-1}; UV λ_{max} 236 nm (ϵ 23 300), 268 (19 100); ^1H NMR 2.37–3.04 (m, 2 H, H-2′), 3.43 (br d, 2 H, J = 5.1 Hz, H-5′), 3.76 (s, 6 H, $2OCH_3$), 4.24 (br d, 1 H, J = 3.0 Hz, H-4′), 4.74 (d, 3 H, J = 5.7 Hz, $CH_2CH{=}CH_2$ and H-3′), 5.25 (dd, 1 H, J = 1.5 and 10.2 Hz, *cis*-$CH_2CH{=}CH_2$), 5.37 (dd, 1 H, J = 1.5 and 17.4 Hz, *trans*-$CH_2CH{=}CH_2$), 6.00 (ddt, 1 H, J = 10.2, 17.4, and 5.7 Hz, $CH_2CH{=}CH_2$), 6.52 (t, 1 H, J = 6.0 Hz, H-1′), 6.80 (d, 4 H, J = 9.0 Hz, protons ortho to OCH_3 of DMTr), 7.13–7.50 (m, 9 H, aromatic protons of DMTr), 8.18 (s, 1 H, H-2), 8.73 (s, 1 H, H-8). Anal. ($C_{35}H_{35}N_5O_7$) C, H, N.

Allyl 1-Benzotriazolyl Carbonate (AOCOBT). To a solution of 1-hydroxybenzotriazole (13.5 g, 0.10 mol) and Et_3N (16.7 mL, 0.12 mol) in THF (350 mL) was added with vigorous stirring AOCCl (11.8 mL, 0.11 mol) at 0 °C. After 10 min, the resulting precipitates were removed by filtration and washed with ethyl acetate (30 mL × 3). The filtrate and washings were collected and evaporated to give a solid that was recrystallized from ethyl acetate, furnishing the pure AOCOBT: mp 107–111 °C (19.9 g, 91%); IR (KBr) 1760, 1430, 1390, 1260, 770 cm^{-1}; UV λ_{max} 236 nm (ϵ 13 900), 321 (11 800), 334 (10 000); ^1H NMR 5.07 (dt, 2 H, J = 5.4 and 1.8 Hz, $CH_2CH{=}CH_2$), 5.4–5.7 (m, 2 H, $CH_2CH{=}CH_2$), 6.14 (ddt, 1 H, J = 10.2, 17.1, and 5.4 Hz, $CH_2CH{=}CH_2$), 7.5–8.3 (m, 4 H, aromatic protons). Anal. ($C_{10}H_9N_3O_3$) C, H, N.

N^4-[(Allyloxy)carbonyl]-5′-*O*-(*p*,*p*′-dimethoxytrityl)-2′-deoxycytidine (9). To a suspension of 5′-deoxycytidine hydrochloride (3.09 g, 11.7 mmol) in pyridine (30 mL) was added trimethylsilyl chloride (7.5 mL, 59.1 mmol), and the mixture was stirred at 10 °C for 1.5 h. To this was added AOCOBT (5.14 g, 23.5 mmol), and the mixture was stirred for 24 h. After addition of an aqueous solution saturated with $NaHCO_3$ (50 mL), the resulting mixture was stirred for 30 min, poured into water (100 mL), and then extracted with dichloromethane (30 mL). The organic layer was separated, and the aqueous layer was extracted with a 1:1 mixture of dichloromethane and pyridine (50 mL × 4). The combined organic extracts were dried over $MgSO_4$ and concentrated to dryness in vacuo to give **8** as an oil. The crude **8** was dissolved in pyridine (30 mL), and to this was added DMTrCl (3.96 g, 11.7 mmol). The mixture was stirred at 10 °C for 24 h. The reaction mixture was poured into water (300 mL) and extracted with a 1:1 mixture of hexane and ethyl acetate (100 mL × 3). The combined organic layers were washed with brine, dried, and concentrated. The crude product was subjected to silica gel column chromatography [silica gel (100 g), $CHCl_3$–MeOH (50:1)] to give **9**: mp 98–102 °C (4.97 g, 69%); IR (KBr) 3400, 2940, 1750, 1650, 1630, 1610, 1560, 1510, 1255, 1210, 1100, 1040 cm^{-1}; UV λ_{max} 237 nm (ϵ 30 600), 285 (8400); ^1H NMR 2.28 (m, 1 H, H-2′), 2.81 (m, 1 H, H-2′), 3.46 (br, 2 H, H-5′), 3.80 (s, 6 H, $2OCH_3$), 4.21 (m, 1 H, H-4′), 4.40–4.80 (m, 3 H, $CH_2CH{=}CH_2$ and H-3′), 5.15–5.51 (m, 2 H, $CH_2CH{=}CH_2$), 5.96 (m, 1 H, $CH_2CH{=}CH_2$), 6.32 (t, 1 H, J = 5.7 Hz, H-1′), 6.70–7.55 (m, 14 H, H-5 and aromatic protons of DMTr), 8.27 (d, 1 H, J = 7.5 Hz, H-6). Anal. ($C_{34}H_{35}N_3O_8$) C, H, N.

N^2-[(Allyloxy)carbonyl]-3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (11). To a solution of 3′,5′-bis-(*O*-*tert*-butyldimethylsilyl)-2′-deoxyguanosine (**10**)[13] (3.40 g, 6.86 mmol) in THF (70 mL) and HMPA (10 mL) was added dropwise a 1.27 M solution of *tert*-butylmagnesium chloride in THF (16.0 mL, 20.3 mmol), and the mixture was stirred at 25 °C for 20 min. To this solution was added AOCCl (1.80 mL, 17.0 mmol) in THF (10 mL), and the resulting mixture was stirred for 15 min. Quenching with methanol (5 mL) and evaporation gave an oil, which was dissolved in ether (200 mL). The solution was washed with an aqueous solution of 0.15 M EDTA (150 mL) followed by saturated aqueous $NaHCO_3$ (50 mL) and brine (50 mL) and then dried. Concentration gave a gummy material that was subjected to silica gel (100 g) column chromatography and eluted with a mixture of hexane and ethyl acetate (3:2) to give **11** (3.09 g, 78%): IR (KBr) 3250, 2950, 2930, 2850, 1715, 1690, 1610, 1240, 1100 cm^{-1}; UV λ_{max} 257 nm (ϵ 14 700), 280 (sh); ^1H NMR 0.06, 0.08 [2 s's, 12 H, $2Si(CH_3)_2$], 0.90 (s, 18 H, 2Si-*t*-C_4H_9), 1.90 (m, 1 H, H-2′), 2.40 (m, 1 H, H-2′), 3.76 (d, 2 H, J = 3.6 Hz, H-5′), 3.98 (dd, 1 H, J = 3.6 and 6.6 Hz, H-4′), 4.57 (dd, 1 H, J = 3.9 and 7.5 Hz, H-3′), 4.73 (d, 2 H, J = 6.0 Hz, $CH_2CH{=}CH_2$), 5.32 (dd, 1 H, J = 1.8 and 10.5 Hz, *cis*-$CH_2CH{=}CH_2$), 5.37 (dd, 1 H, J = 1.8 and 17.4 Hz, *trans*-$CH_2CH{=}CH_2$), 5.94 (ddt, 1 H, J = 10.5, 17.4, and 6.0 Hz, $CH_2CH{=}CH_2$), 6.23 (t, 1 H, J = 6.3 Hz, H-1′), 7.95 (s, 1 H, H-8).

N^2-[(Allyloxy)carbonyl]-5′-*O*-(*p*,*p*′-dimethoxytrityl)-2′-deoxyguanosine (13). To a solution of **11** (2.20 g, 3.79 mmol) in THF (20 mL) was added a 1.0 M solution of TBAF in THF (20 mL, 20 mmol), and the resulting mixture was stirred at 10 °C for 30 min. After concentration, water (20 mL) was added to the resulting viscous residue, and the mixture was extracted with a 1:1 mixture of dichloromethane and pyridine (50 mL × 3). The organic layer was dried over $MgSO_4$. After concentration to dryness in vacuo, the resulting residue **12** was dissolved in pyridine (30 mL), and to this solution was added DMTrCl (1.32 g, 3.90 mmol). After 15 h of stirring at 10 °C, the reaction mixture was poured into water (300 mL) and extracted with a 1:1 mixture of hexane and ethyl acetate (100 mL × 3). The combined organic layer was dried and concentrated. The crude product was subjected to silica gel (70 g) column chromatography and eluted with a mixture of $CHCl_3$ and MeOH (30:1) to afford **13**: mp 124–127 °C (1.62 g, 65%); IR (KBr) 3400, 3250, 2970, 2940, 1700, 1615, 1570, 1515, 1250, 1180, 1100, 1040 cm^{-1}; UV λ_{max} 234 nm (ϵ 33 300), 276 (sh); ^1H NMR 2.47–2.90 (m, 2 H, H-2′), 3.40 (br, 2 H, H-5′), 3.72 (s, 6 H, $2OCH_3$), 4.28 (m, 1 H, H-4′), 4.80 (br d, 2 H, J = 4.8 Hz, $CH_2CH{=}CH_2$), 5.05 (m, 1 H, H-3′), 5.18–5.53 (m, 2 H, $CH_2CH{=}CH_2$), 5.74–6.40 (m, 2 H, $CH_2CH{=}CH_2$ and H-1′), 6.74 (d, 4 H, J = 9.9 Hz, protons ortho to OCH_3 of DMTr), 7.02–7.53 (m, 9 H, aromatic protons of DMTr), 7.77 (s, 1 H, H-8). Anal. ($C_{35}H_{35}N_5O_8$) C, H, N.

(Allyloxy)bis(diisopropylamino)phosphine (22). To a solution of diisopropylamine (130 mL, 0.928 mol) in ether (450 mL) was added dropwise a solution of allyl phosphorodichloridite (36.0 g, 0.227 mol) in ether (30 mL) at −15 °C. The mixture was brought to 25 °C and stirred for 20 h. After removal of the resulting amine hydrochloride salts by filtration, the filtrate was concentrated under a reduced pressure and distilled (0.4 Torr, 114–117 °C) to give **22** (33.9 g, 52%): IR ($CHCl_3$) 2970, 1360, 1185, 1025, 955 cm^{-1}; ^1H NMR 1.17 [dd, 24 H, J = 1.5 and 6.6 Hz, $4CH(CH_3)_2$], 3.55 [sept d, 4 H, J = 6.6 and 10.5 Hz, $4CH(CH_3)_2$], 4.11 (ddt, 2 H, J = 5.1, 7.5, and 1.8 Hz, $CH_2CH{=}CH_2$), 4.99–5.43 (m, 2 H, $CH_2CH{=}CH_2$), 5.98 (ddt, 1 H, J = 10.2, 17.4, and 5.1 Hz, $CH_2CH{=}CH_2$); ^{31}P NMR 125.7. Anal. ($C_{15}H_{33}N_2OP$) C, H, N.

N^6-[(Allyloxy)carbonyl]-5′-*O*-(*p*,*p*′-dimethoxytrityl)-2′-deoxyadenosine 3′-(Allyl *N*,*N*-diisopropylphosphoramidite) (15). To a solution of **7** (3.03 g, 4.72 mmol) in CH_3CN (30 mL) were added diisopropylamine (0.73 mL, 5.21 mmol), **22** (2.2 mL, 7.18 mmol), and 1*H*-tetrazole (366 mg, 5.23 mmol). After 1.7 h of stirring at 25 °C, the mixture was poured into ethyl acetate (150 mL), washed with brine (20 mL × 2), and dried over $MgSO_4$. The resulting solution was concentrated to give a gummy material, which was dissolved in benzene (20 mL). The solution was poured into pentane (300 mL) at −78 °C with stirring to give a powder, which was collected through a glass filter. The resulting cake was dissolved in ethyl acetate and then the solution was evaporated in vacuo to afford **15**: mp 52–55 °C (3.58 g, 91%); IR ($CHCl_3$) 3000, 2970, 1760, 1610, 1510, 1460, 1250 cm^{-1}; UV λ_{max} 237 nm (ϵ 19 400), 268 (16 300); ^1H NMR 1.23 [d, 12 H, J = 7.2 Hz, $2CH(CH_3)_2$], 2.47–3.12 (m, 2 H, H-2′), 3.34–3.83 [m, 10 H, $2CH(CH_3)_2$, $2OCH_3$, and H-5′], 4.00–4.61 (m, 3 H, $POCH_2CH{=}CH_2$ and H-4′), 4.79 (d, 2 H, $COOCH_2CH{=}CH_2$), 5.03–5.54 (m, 5 H, $POCH_2CH{=}CH_2$, $COOCH_2CH{=}CH_2$, and H-3′), 5.70–6.26 (m, 2 H, $POCH_2CH{=}CH_2$ and $COOCH_2CH{=}CH_2$), 6.53 (t, 1 H, J = 6.3 Hz, H-1′), 6.83 (d, 4 H, J = 9.0 Hz, protons ortho to OCH_3 of DMTr), 7.16–7.52 (m, 9 H, aromatic protons of DMTr), 8.20, 8.23 (2 s's, 1 H, H-2 of two diastereomers), 8.64–8.80 (br, 2 H, NHCO and H-8); ^{31}P NMR 148.4. Anal. Calcd for $C_{44}H_{53}N_6O_8P$: C, 64.05; H, 10.19; N, 6.49. Found: C, 63.39; H, 6.84; N, 10.83.

N^6-[(Allyloxy)carbonyl]-5′-*O*-(*p*,*p*′-dimethoxytrityl)-2′-deoxycytidine 3′-(Allyl *N*,*N*-diisopropylphosphoramidite) (16). To a solution of **9** (4.52 g, 7.34 mmol) in CH_3CN (50 mL) were added diisopropylamine (1.04 mL, 7.42 mmol), **22** (2.67 mL, 8.71 mmol), and 1*H*-tetrazole (520 mg, 7.43 mmol). After 3 h of stirring at 25 °C, the mixture was poured into ethyl acetate (200 mL), washed with brine (50 mL × 2), and dried over $MgSO_4$. A similar workup as in the case of **15** afforded **16**: mp 68–71 °C (5.18 g, 88%); IR ($CHCl_3$) 2970, 1750, 1660, 1510, 1490, 1250 cm^{-1}; UV λ_{max} 237 nm (ϵ 25 500), 285 (8100); ^1H NMR 1.20 [d, 12 H, J = 7.2 Hz, $2CH(CH_3)_2$], 2.22 (m, 1 H, H-2′), 2.80 (m, 1 H, H-2′), 3.36–3.87 [m, 10 H, $2OCH_3$, $2CH(CH_3)_2$, and H-5′], 3.93–4.33 (m, 2 H, $POCH_2CH{=}CH_2$), 4.40–4.79 (m, 3 H, $COOCH_2CH{=}CH_2$ and H-4′), 5.00–5.52 (m, 5 H, $POCH_2CH{=}CH_2$, $COOCH_2CH{=}CH_2$, and H-3′), 5.67–6.15 (m, 2 H, $POCH_2CH{=}CH_2$ and $COOCH_2CH{=}CH_2$), 6.31 (dt, 1 H, J = 2.4 and 6.4 Hz, H-1′), 6.90 (d, 5 H, J = 9.3 Hz, protons ortho to OCH_3 of DMTr and H-5), 7.23–7.55 (m, 9 H, aromatic protons of DMTr), 7.80 (br, 1 H, NHCO), 8.31 (dd, 1 H, J = 4.8 and 7.8 Hz, H-6); ^{31}P NMR 148.3, 149.0. Anal. ($C_{43}H_{53}N_4O_9P$) H, N. C: calcd, 64.48; found, 63.45.

N^2-[(Allyloxy)carbonyl]-5′-*O*-(*p*,*p*′-dimethoxytrityl)-2′-deoxy-

guanosine 3′-(Allyl *N*,*N*-diisopropylphosphoramidite) (17). To a solution of **13** (2.54 g, 3.86 mmol) in CH_3CN (25 mL) were added diisopropylamine (0.52 mL, 3.71 mmol), **22** (1.3 mL, 4.24 mmol), and 1*H*-tetrazole (260 mg, 3.71 mmol). After 2 h of stirring at 25 °C, the mixture was poured into ethyl acetate (150 mL), washed with brine, and dried over $MgSO_4$. A similar workup as above afforded **17**: mp 66–72 °C (2.77 g, 85%); IR ($CHCl_3$) 1710, 1610, 1470, 1390, 1100 cm^{-1}; UV λ_{max} 238 nm (ϵ 27 500), 274 (sh); ^1H NMR 1.12 [d, 12 H, J = 6.0 Hz, $2CH(CH_3)_2$], 2.26–2.95 (m, 2 H, H-2′), 3.20–3.80 [m, 10 H, $2CH(CH_3)_2$, $2OCH_3$, and H-5′], 3.90–4.35 (m, 3 H, $POCH_2CH{=}CH_2$ and H-4′), 4.64 (d, 2 H, J = 6.0 Hz, $COOCH_2CH{=}CH_2$), 4.95–5.42 (m, 5 H, $POCH_2CH{=}CH_2$, $COOCH_2CH{=}CH_2$, and H-3′), 5.60–6.27 (m, 3 H, $POCH_2CH{=}CH_2$, $COOCH_2CH{=}CH_2$, and H-1′), 6.65–6.85 (m, 4 H, protons ortho to OCH_3 of DMTr), 7.10–7.47 (m, 13 H, aromatic protons of DMTr), 7.73, 7.75 (2 s's, 1 H, H-8 of two diastereomers); ^{31}P NMR 148.2. Anal. ($C_{44}H_{53}N_6O_9P$) C, H, N.

5′-*O*-(*p*,*p*′-Dimethoxytrityl)thymidine 3′-(Allyl *N*,*N*-diisopropylphosphoramidite) (18). To a suspension of 5′-*O*-(*p*,*p*′-dimethoxytrityl)-thymidine (**14**)[14] (5.05 g, 9.25 mmol) in CH_3CN (60 mL) were added diisopropylamine (1.43 mL, 10.2 mmol), **22** (4.3 mL, 14.0 mmol), and 1*H*-tetrazole (715 mg, 10.2 mmol). After 1.5 h of stirring at 25 °C, the mixture was poured into ethyl acetate (300 mL), washed with brine, and dried over $MgSO_4$. The mixture was similarly worked up as above to afford **18**: mp 67–70 °C (6.84 g, 100%); IR ($CHCl_3$) 1690, 1510, 1250, 1180, 980 cm^{-1}; UV λ_{max} 234 nm (ϵ 21 500), 269 (11 000); ^1H NMR 1.23 [dd, 12 H, J = 6.3 and 9.0 Hz, $2CH(CH_3)_2$], 1.42 (s, 3 H, CH_3), 2.10–2.70 (m, 2 H, H-2′), 3.34–3.85 [m, 10 H, $2CH(CH_3)_2$, $2OCH_3$, and H-5′], 3.90–4.27 (m, 3 H, $CH_2CH{=}CH_2$ and H-4′), 4.60 (m, 1 H, H-3′), 4.97–5.49 (m, 2 H, $CH_2CH{=}CH_2$), 5.91 (m, 1 H, $CH_2CH{=}CH_2$), 6.44 (m, 1 H, H-1′), 6.85 (d, 4 H, J = 9.0 Hz, protons ortho to OCH_3 of DMTr), 7.20–7.53 (m, 13 H, aromatic protons of DMTr), 7.66 (m, 1 H, H-6), 8.36 (br, 1 H, NHCO); ^{31}P NMR 148.0, 148.5. Anal. ($C_{40}H_{50}N_3O_8P$) N. C: calcd, 65.64; found, 65.10. H: calcd, 6.90; found, 7.32.

Deprotection of Oligodeoxyribonucleotides. (a) Allyl–AOC Method. The CPG supports binding the protected oligomer were washed with THF (1.0 mL) and dried in vacuo. To the resulting polymer were added $P(C_6H_5)_3$, $Pd_2(dba)_3$–$CHCl_3$, and a 1.2 M solution of n-$C_4H_9NH_2$–HCOOH (1:1) in THF. After being vigorously shaken with a mixer, the mixture was heated at 50 °C for 0.5–1 h. After the supernatant fluid was decanted, the resulting CPG supports were washed successively with THF (1 mL × 2), acetone (1 mL × 2), a 0.1 M sodium *N*,*N*-diethyldithiocarbamate (ddtc) aqueous solution (pH 9.7, 1 mL, 15 min), acetone (1 mL × 3), and water (1 mL). The ddtc washing was repeated once more. The cleaned CPG supports were treated with concentrated NH_4OH (1.5 mL) at 25 °C for 2 h to afford the fully deprotected oligoDNA. Reaction of the protected 32mer with $Pd_2(dba)_3$–$CHCl_3$ (140 mg, 0.135 mmol), $P(C_6H_5)_3$ (360 mg, 1.37 mmol), and n-$C_4H_9NH_2$–HCOOH in THF (6.7 mL, 8.04 mmol) gave 182 OD_{260} of crude **1**. Reaction of the protected 43mer with $Pd_2(dba)_3$–$CHCl_3$ (190 mg, 0.184 mmol), $P(C_6H_5)_3$ (490 mg, 1.87 mmol), and n-$C_4H_9NH_2$–HCOOH in THF (9.1 mL, 10.9 mmol) gave 256 OD_{260} of crude **2**. Reaction of the protected 60mer (0.58 μmol) with $Pd_2(dba)_3$–$CHCl_3$ (156 mg, 0.151 mmol), $P(C_6H_5)_3$ (400 mg, 1.53 mmol), and n-$C_4H_9NH_2$–HCOOH in THF (7.4 mL, 8.88 mmol) gave 209 OD_{259} of crude **3**.

(b) Conventional Method. To the CPG supports binding the protected oligoDNA was added concentrated NH_4OH (1.5 mL), and the mixture was allowed to stand at 25 °C for 2 h. The supernatant fluid was separated from the CPG supports, which were washed with concentrated NH_4OH (0.5 mL). The combined aqueous solution was heated at 55 °C for 12 h to afford the fully deprotected material. Reactions by the conventional method afforded 174 OD_{260} of **1**, 218 OD_{260} of **2**, and 368 OD_{259} of **3** as crude materials, respectively.

Purification of Fully Deprotected Oligodeoxyribonucleotides. To the crude DNA oligomer (ca. 30 OD_{260}) synthesized by the allyl–AOC method in H_2O (8 μL) was added loading buffer (3 μL) containing 0.08% bromophenol blue (BPB), 0.1% xylene cyanol (XC), and 20 mM EDTA in deionized formamide. The mixture was loaded onto a 12% polyacrylamide/8 M urea gel (1.5 mm × 20 cm × 40 cm) and run at 800 V for 3.5 h. After electrophoresis, the gel was covered with Saran wrap and illuminated with an UV lamp over an intensifying screen. The desired band was excised, placed into an 11-mL polypropylene Econo column (Bio-Rad), cut into small pieces, and covered with an aqueous solution (1 mL) of 10 mM Tris-HCl (pH 8.0), 0.1% sodium dodecyl sulfate (SDS), and 1 mM EDTA. The column was kept at 37 °C overnight. The supernatant was collected by centrifugation to afford pure oligoDNA, which was desalted by ethanol precipitation to give the pure sample. The isolated yields are summarized in Table II.

HPLC Analysis of the Enzymatic Digestion Products. A mixture of the purified oligoDNA (0.2–1.5 OD_{260}) synthesized by the allyl–AOC method in H_2O (31.5 μL), snake venom phosphodiesterase (SVP) (1 μL, 0.1 unit), bacterial alkaline phosphatase (BAP) (10 μL, 2.5 units), 300 mM Tris-HCl (5 μL, final concentration 30 mM), and 300 mM $MgCl_2$ (2.5 μL, final concentration 15 mM) was incubated at 37 °C for 12 h and then heated at 90 °C for 0.5 min. The aliquots (5 μL) were injected directly onto an ODS-Wakosil column and eluted with H_2O–MeOH (9:1) or H_2O–MeOH (6:4) (1 mL/min, 40 °C). The ratio of deoxyribonucleosides was determined by the usual method [dC, t_R = 6.7 min; dG, 9.4 min; T, 12.3 min; dA, 17.2 min; eluted with H_2O–MeOH (9:1)]. Anal. Found for **1**: dC, 6.8; dG, 2.7; T, 9.0; dA, 12.7. Calcd: dC, 7; dG, 3; T, 9; dA, 13. Anal. Found for **2**: dC, 12.9; dG; 6.4; T; 11.0; dA, 12.8. Calcd: dC, 13; dG, 6; T, 11; dA, 13. Anal. Found for **3**: dC, 12.5; dG, 12.6; T, 15.0: dA, 20.5. Calcd: dC, 12; dG, 13; T, 15, dA, 20. No peaks were detected in the area eluting the *N*-AOC-protected nucleosides [t_R = 10–12 min; eluted with H_2O–MeOH (6:4)].

Preparation of ^{32}P-Labeled 5′-Monophosphates of the OligoDNAs and Bio-Image Analysis. An aqueous solution of an unpurified oligoDNA (0.003 OD_{260}) was diluted to 8 μL with a TE solution [a mixture of 10 mM Tris-HCl (pH 7.5) and 1 mM EDTA in H_2O]. To the solution were added 5′-[γ-^{32}P]ATP (specific activity 300 Ci/mmol, concentration 30.9 pmol/μL) (0.625 μL, 19.3 pmol, 5.8 μCi), 375 μM ATP (0.082 μL, 30.8 pmol), T4 polynucleotide kinase (1 μL, 8 units), 10× kinase buffer [a mixture of 0.5 M Tris-HCl (pH 7.6), 0.1 M $MgCl_2$, 50 mM dithiothreitol, 1 mM spermidine hydrochloride, and 1 mM EDTA (pH 7.6) in H_2O] (1 μL), and H_2O (0.293 μL), and then the mixture was incubated at 37 °C for 30 min. A 1-μL aliquot of the reaction mixture was soaked into a Whatman DE 81 paper disk, and then the unreacted ATP was eluted with an aqueous solution of 0.3 M $HCOONH_4$–10 mM $Na_4P_2O_7$–0.1% SDS. Analysis of the remaining sample in the paper disk by liquid scintillation counting indicated that the labeled sample was obtained. To 3 μL of the solution was added deionized formamide (6 μL) containing 0.08% BPB, 0.1% XC, and 20 mM EDTA. After the mixture was heated at 90 °C for 0.5 min, 1.5 μL of this solution was loaded onto a 12% polyacrylamide/8 M urea gel (0.5-mm thickness, 40-cm length, 1300 V) and run until the BPB had migrated approximately 20 cm from the wells. After electrophoresis, one glass plate was removed from the polyacrylamide slab and the gel was covered with Saran wrap. The bio-image chromatogram (Figure 1) was obtained by exposing the gel to an imaging plate at 25 °C for 1 h and then analyzing with the aid of a BA100 bio-image analyzer of Fuji Photo Film Co., Ltd.

Atomic Absorption Spectrometry of Oligodeoxyribonucleotides. Atomic absorption spectrometry of the residual palladium in an aqueous solution of oligoDNA was carried out at 247.5 nm by the flameless method using a Perkin-Elmer 603 atomic absorption spectrometer and a Hitachi Hollow cathode lamp palladium. The approximate quantity of residual palladium was determined by the usual method using palladium standard solutions.

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