Facile Synthesis of Oligodeoxyribonucleotides via the Phosphoramidite Method without Nucleoside Base Protection

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Abstract: A facile synthesis of oligodeoxyribonucleotides via the phosphoramidite approach without base protection of the building blocks has been developed; it relies on the use of imidazolium triflate as a promoter for the condensation of a nucleoside phosphoramidite and a nucleoside. In the solution phase, the condensation is accomplished in a highly O-selective manner by using equimolar amounts of an N-free nucleoside phosphoramidite and an N-unblocked nucleoside to give, after oxidation with bis(trimethylsilyl)peroxide or with *tert*-butyl hydroperoxide, a dinucleoside phosphate in >95% yield. In the solid-phase synthesis, which requires an excess amount of the phosphoramidite for the condensation, deoxyadenosine and deoxycytidine undergo N-phosphitylation to some extent. The undesired product, however, can be converted to the N-free derivative by brief treatment with benzimidazolium triflate in methanol. Thus the overall process allows the chemoselective formation of internucleotide linkage. The oligomers prepared by this N-unprotected solid-phase approach include $^{5'}$GTCACGACGTTGTAAAACGAC$^{3'}$ (21mer), $^{5'}$CAGGAAACAG-CTATGACCATG$^{3'}$ (21mer), $^{5'}$CAAGTTGATGAACAATACTTCATACCTAAACT$^{3'}$ (32mer), and $^{5'}$TATGGGCCTTTTGATAG-GATGCTCACCGAGCAAAACCAAGAACAA-CCAGGAGATTTTATT$^{3'}$ (60mer), which are provided in excellent quality. PCR amplification of DNAs using the crude 21mers as primers is also demonstrated.

Introduction

Synthesis of oligodeoxyribonucleotides is an important component in nucleic acid researches,[1] and the phosphoramidite method is currently seeing the most use.[1a,b,2−4] This method includes the condensation of a nucleoside phosphoramidite and a nucleoside; in this key step, NH_2 moieties of nucleoside bases have universally been protected to prevent the undesired *N*-phosphitylation in the conventionally employed synthetic methods.[1b] As Letsinger has stated,[5] ideally it would be best to avoid the N-protecting groups because they entail at least two additional steps, introduction and removal, and the reagents required in these steps limit the range of functional groups that can be tolerated in the synthesis. Further, the approach without nucleoside base protection (the N-unprotected method) may considerably diminish the risk of depurination of deoxyadenosine[1b,6,7,8] [$t_{1/2}$ (2% dichloroacetic acid in dichloromethane, 25 °C) = ca. 5 h],[6] which is a serious problem in the N-protected method, particularly using the building blocks with acyl protectors such as benzoyl ($t_{1/2}$ = ca. 5 min)[6] and phenoxyacetyl (PAC) ($t_{1/2}$ = ca. 25 min).[9] To solve these problems, Letsinger recently reported a pioneering DNA synthesis with N-unprotected building blocks which employs a mixture of pyridinium hydrochloride and aniline[5] or imidazole[10] as the activator of the phosphoramidites.[11,12] However, this method has some drawbacks and limitations, e.g., the pyridinium salt is very hygroscopic and thus not suitable for a synthesis requiring anhydrous conditions. Further, the mixed activator is useful for some limited phosphoramidites such as methyl *N*,*N*-diisopropylphosphoramidites but not quite effective for widely employed 2-cyanoethyl *N*,*N*-diisopropylphosphoramidites.[3a] In

(1) For comprehensive reviews on oligonucleotide synthesis, see: (a) Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1993**, *49*, 6123−6194. (b) Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1992**, *48*, 2223−2311. (c) Caruthers, M. H. *Science* **1985**, *230*, 281−285. (d) Hata, T.; Matsuzaki, J. *J. Synth. Org. Chem., Jpn.* (in Japanese) **1984**, *42*, 429−436. (e) Narang, S. A. *Tetrahedron* **1983**, *39*, 3−22. (f) Ikehara, M.; Ohtsuka, E.; Markham, A. F. *Adv. Carbohydr. Chem. Biochem.* **1979**, *36*, 135−213. (g) Reese, C. B. *Tetrahedron* **1978**, *34*, 3143−3179. (h) Amarnath, V.; Broom, A. D. *Chem. Rev.* **1977**, *77*, 183−217. (i) Zhdanov, R. I.; Zhenodarova.

(2) Hayakawa, Y.; Kataoka, M.; Noyori, R. *J. Org. Chem.* **1996**, *61*, 7996−7997.

(3) (a) Sinha, N. D.; Biernat, J.; Köster, H. *Tetrahedron Lett.* **1983**, *24*, 5843−5846. (b) McBride, L. J.; Caruthers, M. H. *Ibid.* **1983**, *24*, 245−248. (c) Adams, S. P.; Kavka, K. S.; Wykes, E. J.; Holder, S. B.; Galluppi, G. R. *J. Am. Chem. Soc.* **1983**, *105*, 661−663. (d) Matteucci, M. D.; Caruthers, M. H. *Ibid.* **1981**, *103*, 3185−3191. (e) Beaucage, S. L.; Caruthers, M. H. *Tetrahedron Lett.* **1981**, *22*, 1859−1862.

(4) (a) Pon, R. T. *Tetrahedron Lett.* **1987**, *28*, 3643−3646. (b) Froehler, B. C.; Matteucci, M. D. *Ibid.* **1983**, *24*, 3171−3174.

(5) Gryaznov, S. M.; Letsinger, R. L. *J. Am. Chem. Soc.* **1991**, *113*, 5876−5877.

(6) Froehler, B. C.; Matteucci, M. D. *Nucleic Acids Res.* **1983**, *11*, 8031−8036.

(7) (a) Vu, H.; McCollum, C.; Jacobson, K.; Theisen, P. *Tetrahedron Lett.* **1990**, *31*, 7269−7272. (b) McBrid, L. J.; Kierzek, R.; Beaucage, S. L.; Caruthers, M. H. *J. Am. Chem. Soc.* **1986**, *108*, 2040−2048.

(8) Schulhof, J. C.; Molko, D.; Teoule, R. *Nucleic Acids Res.* **1987**, *15*, 397−416.

(9) A result obtained in our laboratory.

(10) Gryaznov, S. M.; Letsinger, R. L. *Nucleic Acids Res.* **1992**, *20*, 1879−1882.

(11) Solution-phase synthesis of dinucleoside phosphates via the phosphoramidite approach, using N-unprotected nucleoside phosphoramidites and nucleosides as building blocks and *N*-methylanilinium trifluoroacetate or trichloroacetate as the promoter, was also reported in the following: Fourrey, J.-L.; Varenne, J. *Tetrahedron Lett.* **1985**, 2663−2666.

(12) There are a number of methods for the synthesis of oligodeoxyribonucleotides using N-unprotected building blocks, as follows. The *H*-phosphonate method: (a) Wada, T.; Sato, Y.; Honda, F.; Kawahara, S.; Sekine, M. *J. Am. Chem. Soc.* **1997**, *119*, 12710−12721. (b) Kung, P. P.; Jones, R. A. *Tetrahedron Lett.* **1992**, 33, 5869−5872. The hydroxyl-activation method: (c) Uchiyama, M.; Aso, Y.; Noyori, R.; Hayakawa, Y. *J. Org. Chem.* **1993**, *58*, 373−379 and references therein. (d) Noyori, R.; Uchiyama, M.; Nobori, T.; Hirose, M.; Hayakawa, Y. *Aust. J. Chem.* **1992**, *45*, 205−225.

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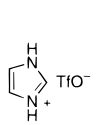
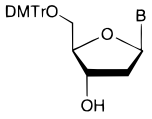
addition, the yield of phosphitylation is not sufficiently high for the synthesis of long oligonucleotides. We report here that imidazolium triflate (**1**)[13] eliminates these drawbacks, serving as a more effective promoter in both solution-phase and solid-phase syntheses of oligodeoxyribonucleotides via the phosphoramidite method without nucleoside base protection.

Results and Discussion

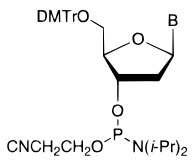
The key reagent **1**, mp 197−198 °C, was quantitatively prepared by mixing imidazole and trifluoromethanesulfonic acid in equimolar amounts in dichloromethane at ambient temperature or in a 1:1 mixture of methanol and ether at 0 °C. This reagent has good solubility in acetonitrile, >1.0 mol/L, and high stability when stored at ambient temperature under atmospheric conditions.



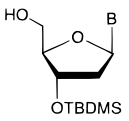
1



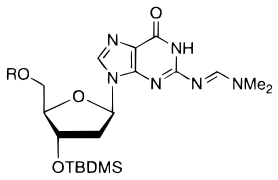
2, B = Ade
3, B = Cyt
4, B = Gua
5, B = Thy



6, B = Ade
7, B = Cyt
8, B = Gua
9, B = Thy
10, B = Ade^{PAC}
11, B = Cyt^{ibu}
12, B = Cyt^{Bz}
13, B = Gua^{PAC}



14, B = Ade
15, B = Cyt
16, B = Thy



17, R = H
18, R = DMTr

N-free nucleoside 3′-phosphoramidites **6**−**9** as monomer units requisite for the synthesis were prepared as follows. Reaction of 2′-deoxyadenosine and *p*,*p*′-dimethoxytrityl chloride (DMTrCl) (1.00 equiv) in the presence of dichloroacetic acid and triethylamine in pyridine (25 °C, 4 h) gave **2** in 77% yield,[14] which was then condensed with $(CNCH_2CH_2O)[(i\text{-}C_3H_7)_2N]PCl$ in THF by the aid of ethyldiisopropylamine[15] (−78 to 25 °C, 60 min) to afford **6** in >95% yield (>73% overall yield). Preparation of the deoxycytidine and thymidine analogues **7** and **9** were also performed by starting from the parent nucleosides via the two-step process shown in the preparation of **6**, in which the yields of tritylation giving **3**[16] and **5**[17] were 70 and 85%, respectively, and those of the amidite formation were >95% in both cases (>66 and 81% overall yields, respectively). In 2′-deoxyguanosine, the 5′-O-selective dimethoxytritylation of the parent 2′-deoxyguanosine could not be directly achieved, and accordingly, the production of the phosphoramidite **8** required N-protection by the amidine group. Thus, at first, 2′-deoxyguanosine was converted to **17** (96% yield) by the treatment with (dimethoxy)(dimethylamino)methane in methanol (50 °C, 15 h).[7] Subsequently, **17** was successively reacted with DMTrCl in pyridine (25 °C, 60 min), affording **18** (68% yield), and with aqueous ammonia and pyridine to give **4** (97% yield). Finally, coupling of **4** with $(CNCH_2CH_2O)[(i\text{-}C_3H_7)_2N]PCl$ in THF by the assistance of ethyldiisopropylamine (−78 to 25 °C, 60 min) provided **8** in 97% yield (63% overall yield from the starting material). In these preparations, the introduction of the phosphoramidite function to the 3′-O-free precursor was also achieved in >95% isolated yield by the reaction with $(CNCH_2CH_2O)P[N(i\text{-}C_3H_7)_2]_2$ (1.10 equiv) promoted by **1** (0.30 equiv) in dichloromethane (25 °C, 60 min). These amidites were obtained as a colorless solid with >95% purity by trituration of the crude products from petroleum ether. The structures of **6**−**9** were confirmed by the ^{31}P NMR signals appearing δ 149−150 ppm (H_3PO_4 standard) (cf. the ^{31}P NMR signals due to *N*-phosphoramidityl products: δ 123−127 ppm). The stability of the N-free compounds is similar to that of N-protected derivatives, and no decrease in their quality was observed by storage for several months under normal conditions. The N-unprotected nucleoside phosphoramidites **6**−**9** could be obtained generally via a shorter pathway and in higher yields than the *N*-acyl-protected analogues employed in conventional methods.[18]

The reagent **1** is an excellent promoter[20] that allows rapid and highly chemoselective condensation of the phosphoramidite and the N-free nucleoside **14**, **15**, or **16** when equimolar amounts of these substrates are employed in a solution phase. For example, the reaction of **6** and **14** in acetonitrile was accomplished within 5 min to give, after oxidation with bis-

(13) Hayakawa, Y.; Kataoka, M. Presented at the International Conference on Nucleic Acids and Related Macromolecules: Synthesis, Structure, Function and Applications, Ulm, Germany, September 1997; Paper PL14.

(14) Ti, G. S.; Gaffney, B. L.; Jones, A. R. *J. Am. Chem. Soc.* **1982**, *104*, 1316−1319.

(15) Sina, N. D.; Biernet, J.; McMarus, J.; Köster, H. *Nucleic Acids Res.* **1884**, *12*, 4539−3557.

(16) Ishido, Y. *Jpn. Kokai Tokkyo Koho* Jp. Patent 01 308 294 [89 308 294], 1989; *Chem. Abstr.* **1990**, *112*, 700.

(17) Schaller, H.; Weimann, G.; Lerch, B.; Khorana, H. G. *J. Am. Chem. Soc.* **1963**, *85*, 3821−3827.

(18) According to previous studies, some representative *N*-acyl-protected nucleoside phosphoramidites are prepared as follows. The deoxyadenosine phosphoramidite with N^4-PAC protection, **10**, was obtained from 2′-deoxyadenosine in 35% overall yield in three steps, i.e., (i) the N-protection using phenoxyacetic anhydride (65% yield), (ii) the 5′-O-protection with DMTrCl (60% yield), and (iii) the phosphoramidite formation with $CNCH_2CH_2O)P[N(i\text{-}C_3H_7)_2]_2$ in the presence of diisopropylammonium tetrazolide (90% yield).[19] In this preparation, the N-protection was also achieved by successive treatments with trimethylsilyl chloride in pyridine, DMTrCl, phenoxyacetyl chloride, and 1-hydroxybenzotriazole (HOBT), and a 1:1:3 mixture of triethylamine, pyridine, and water (65% overall yield).[8] Just as in this latter process, where benzoyl chloride and pyridine are used in place of phenoxyacetyl chloride and HOBT, *N*-benzoyldeoxyadenosine phosphoramidite can be prepared in a higher overall yield[14,15] comparable to that of the preparation of the N-unprotected analogue. However, the benzoylated derivative is rarely used at present due to its tendency to undergo depurination. The preparation of *N*-isobutyryldeoxycytidine phosphoramidite **11** from deoxycytidine is achieved in 32% overall yield via a pathway consisting of (i) the introduction of the isobutyryl protecting group to the nucleoside base by successive treatment in one pot with trimethylsilyl chloride, isobutyryl chloride, and aqueous ammonia (60% yield), (ii) the 5′-*O*-dimethoxytritylation (75% yield), and (iii) the phosphoramiditylation with $(CNCH_2CH_2O)P[N(i\text{-}C_3H_7)_2]_2$ and diisopropylammonium tetrazolide (70% yield).[15] In a similar manner, the *N*-benzolydeoxycytidine phosphoramidite **12** can be synthesized in a higher yield (69% overall yield)[15] comparable to that of **6**, but this derivative is less useful than **11** because removal of the benzoyl protector requires rather harsh basic conditions that cause the cleavage of the internucleotide linkage. The *N*-PAC-protected deoxyguanosine phosphoramidite **13** is obtained from the parent nucleoside in 28% overall yield via a five-step process similar to that employed for the preparation of **11**.[19]

(19) Schulhof, J. C.; Molko, D.; Teoule, R. *Tetrahedron Lett.* **1987**, *28*, 51−54. The monomer building blocks were supplied by Amersham Pharmacia Biotech.

(20) As reported in ref 2, **1** is not effective as a promoter of the condensation using lowly reactive phosphoramidites such as *o*-chlorophenyl phosphoromorpholidites. This salt, however, acts as an excellent activator toward fairly reactive phosphoramidites, such as 2-cyanoethyl *N*,*N*-diisopropylphosphoramidites.

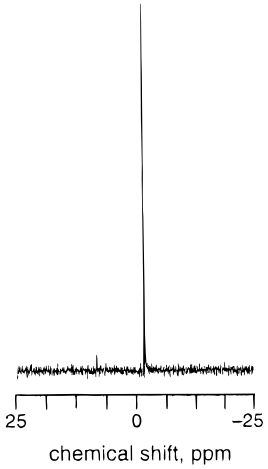
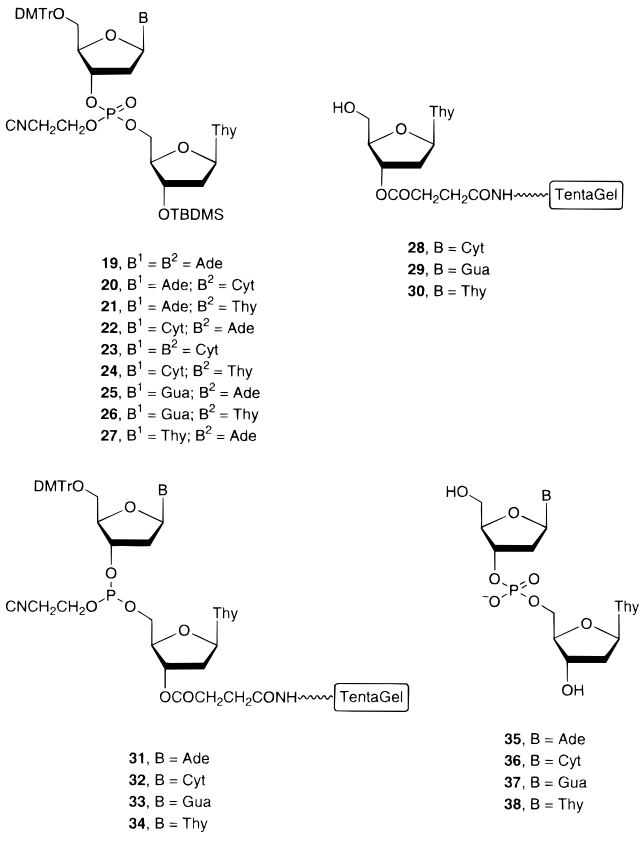


Figure 1. ^{31}P NMR spectrum of crude **20**.

(trimethylsilyl) peroxide[21] or with *tert*-butyl hydroperoxide (TBHP),[21] **19** in 95% isolated yield as a mixture of two



diastereomers. The structure of **19** and purity of the crude material was confirmed by the ^{31}P NMR spectrum, which shows two eminent signals at −1.1 and −1.0 ppm (H_3PO_4 standard), supporting the presence of the diastereomeric phosphotriester functions, but no detectable signal due to *N*-phosphoryl products (Figure 1) (cf. the ^{31}P NMR signals due to *N*-phosphoryl products: δ 0−2 ppm). Such high-yield and selective preparations of dinucleoside phosphates were also achieved with other N-free phosphoramidites and nucleosides. Table 1 lists several examples of the preparation via this method. By contrast, the reaction using excess equivalents of the phosphoramidite and the promoter to the nucleoside, which is a reaction generally

(21) Hayakawa, Y.; Uchiyama, M.; Noyori, R. *Tetrahedron Lett.* **1986**, *27*, 4191−4194.

Table 1. Preparation of Dinucleoside Phosphates in a Solution Phase[a]

amidite	nucleoside	product[b]	yield,[c] %
6	**14**	**19**	95
6	**15**	**20**	93
6	**16**	**21**	95
7	**14**	**22**	96
7	**15**	**23**	95
7	**16**	**24**	94
8	**14**	**25**	96
8	**16**	**26**	98
9	**14**	**27**	98

[a] Condensation was carried out using amidite, nucleoside, and imidazolium triflate in an equimolar ratio in acetonitrile at 25 °C for 5 min. The resulting phosphite was directly oxidized by bis(trimethylsilyl)peroxide with trimethylsilyl triflate as a catalyst in an acetonitrile−CH_2Cl_2 mixture (25 °C, 5 min) to the phosphate. [b] The product was obtained as ca. 1:1 mixture of diastereomers. [c] Isolated yield.

used in the solid-phase synthesis of oligonucleotides, was not achieved with perfect chemoselectivity in the cases using the N-free adenyl phosphoramidite **6** or cytosyl phosphoramidite **7**. Thus, phosphitylation of the thymidine **30**, attached to TentaGel support via the long-chain alkylamine spacer arm, with a 1:1 mixture of **1** and **6** (25 equiv each to **30**) (1 min) gave not only the desired phosphite **31** (^{31}P NMR: δ 140 ppm) but also a small amount (4%) of its *N*-phosphitylated derivative(s) showing ^{31}P NMR signals at δ 123−127 ppm.[22,23] A similar result was obtained in the reaction of **7**, affording ca. 8% of undesired N-phosphitylated product in addition to **32**. However, these *N*-phosphitylated compounds could be quantitatively reverted to the N-free derivative **31** or **32** without cleavage of the internucleotidic phosphite linkage by treatment with benzimidazolium triflate[2] (50 equiv) in methanol at 25 °C for 2 min, while the subsequent treatment with TBHP for oxidation (1 min) to the phosphate,[21] with dichloroacetic acid for detritylation (2 min), and with concentrated ammonia for removal of the cyanoethyl protector and detachment from the solid support (180 min), gave the target compounds **35** and **36** in 94 and 96% isolated yields, respectively. Accordingly, the preparation of nucleotides via the N-unprotected method was considered to have been accomplished in a formal sense. Here, the removal of the *N*-phosphityl group had to be carried out prior to the oxidation, since the *N-phosphoryl* group could not be eliminated by the benzimidazolium triflate−methanol reaction. The phosphitylation of **30** by the guanyl or thyminyl phosphoramidite **8** or **9** under similar conditions proceeded in a perfect O-selective manner to give the phosphites **33** and **34**, showing signals at δ 140−141 ppm. The subsequent oxidation, deprotection, and detachment afforded **37** or **38** in 94 or 95% isolated yield, respectively. The reaction using other promoters, such as benzimidazolium triflate, 1*H*-tetrazole, or 5-(*p*-nitrophenyl)-1*H*-tetrazole, produced a considerable amount of N-phosphitylation of the adenyl and cytosyl derivatives. For example, the reaction of **6** or **7** and **30** with 1*H*-tetrazole gave the *N*-phosphityl product(s) in 70−250% yield. In such cases, the quantitative removal of the *N*-phosphityl group could not be achieved by

(22) The ^{31}P NMR signals suggested formation of *N*-phosphitylated products, but their exact structure was not determined.

(23) When other promoters were used, such as benzimidazolium triflate, 1*H*-tetrazole, and 5-(*p*-nitrophenyl)-1*H*-tetrazole, there was considerable N-phosphitylation of the adenyl and cytosyl derivatives. For example, the reaction of **6** or **7** and **30** with 1*H*-tetrazole gave the *N*-phosphityl product(s) in 70−250% yield. In such cases, the quantitative removal of the *N*-phosphityl group could not be achieved by the brief treatment with benzimidazolium triflate in methanol. Guanyl and thyminyl bases underwent little or no N-phosphitylation even when benzimidazolium triflate, 1*H*-tetrazole, or 5-(*p*-nitrophenyl)-1*H*-tetrazole was used as the promoter.

Table 2. Reaction Sequence of the Solid-Phase Synthesis

step	operation	reagent(s)	time, min
1	washing	CH_3CN	0.3
2	detritylation	3% CCl_3COOH/CH_2Cl_2	2.0
3	washing	CH_3CN	2.0
4	coupling	0.1 M amidite/CH_3CN + 0.1 M IMT[a]/CH_3CN	1.0
5	washing	CH_3CN	0.3
6	N-P cleavage	0.5 M BIT[b]/CH_3OH	2.0
7	washing	CH_3CN	0.6
8	oxidation	1 M *t*-C_4H_9OOH/toluene	0.8
9	washing	CH_3CN	0.3

the brief treatment with benzimidazolium triflate in methanol. Guanyl and thyminyl bases did not undergo N-phosphitylation even when benzimidazolium triflate, 1*H*-tetrazole, or 5-(*p*-nitrophenyl)-1*H*-tetrazole was used as the promoter. No detritylation, depurination or depyrimdination, or oxidation of the nucleoside was observed throughout the entire process. These results confirmed the solid-phase synthesis of oligodeoxyribonucleotides via the phosphoramidite method using N-unprotected building blocks (the unprotected method).

We prepared four kinds of DNA oligomers, 5′GTCAC-GACGTTGTAAAACGAC3′ (**39**) (21mer), 5′CAGGAAACAGC-TATGACCATG3′ (**40**) (21mer), 5′CAAGTTGATGAAC-AATA-CTTCATACCTAAACT3′ (**41**) (32mer),[24] and 5′TATGGGCC-TTTTGATAGGATG-CTCACCGAGCAAAACCAAGAACAA-CCAGGAGATTTTATT3′ (**42**) (60mer).[24]

The synthesis by the unprotected method was performed with **6**−**9** as the monomer units on 1−μmol scale on an Applied Biosystems 381A DNA synthesizer, starting from the suitable solid-anchored nucleoside **29**, **30**, or **31**. Table 2 shows the reaction cycle for the chain elongation which required 10.3 min. Since the dimethoxytrityl cation analysis indicated that the average coupling yield is >99.8% in all the syntheses, the step for capping the unreacted hydroxyl was eliminated in the reaction sequence. After the final condensation, removal of the dimethoxytrityl protector of the 5′-hydroxyl terminus by exposure to dichloroacetic acid in dichloromethane (2 min), followed by the simultaneous deblocking of the cyanoethyl protectors of the internucleotide linkages and detachment of the product from the solid support by treatment with concentrated ammonia (180 min), gave the oligomers **39**−**42**. Isolated yields were 72% for **39**, 74% for **40**, 78% for **41**, and 54% for **42**. The purity of **41** and **42** in the crude forms was analyzed by capillary gel electrophoresis (CGE). With respect to **41**, the purity was compared with that of the crude product obtained by an approach using the *N*-PAC-protected nucleoside phosphoramidite as a monomer unit (the PAC-protected method[8,18]). Figure 2 illustrates CGE profiles of the two kinds of **41** and **42**. The analysis exhibited that **41** produced by the unprotected approach is of an excellent quality that is comparable to that derived from the PAC-protection method (cf. the spectra A and B).[25] The profile of **42** (the spectrum C) also indicated the high purity of this oligomer. The crude **41** was further subjected to mass spectrometry to confirm its purity and structure. The MALDI time-of-flight (TOF) mass spectrum (Figure 3) provided a single

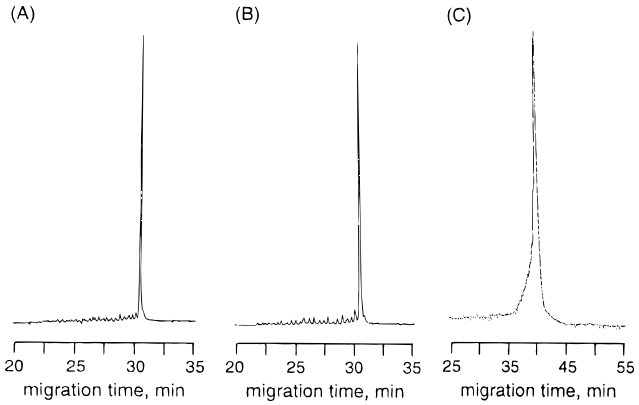


Figure 2. Capillary gel electrophoresis profiles of crude products of **41** and **42**: (A) **41** prepared via the unprotected method; (B) **41** prepared via the PAC-protected method; (C) **42** synthesized by the unprotected method.

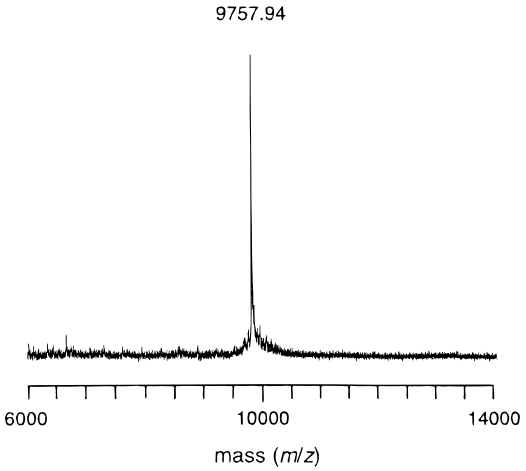


Figure 3. MALDI-TOF mass spectrum of crude **42** synthesized by the unprotected method.

peak at *m*/*z* 9757.94 in the region where the molecular peak appears. This result supported the structure of **41** ($C_{313}H_{394}N_{119}O_{186}P_{31}$), whose calculated molecular weight is 9759.43. The electrospray mass spectrum also suggested that the molecular weight of **41** is 9759.56. These results supported the structure as well as the high purity of **41**, while the same mass spectrometric analyses of **42** gave no eminent peak in the molecular-weight area. Therefore, the purity of **42** was further investigated by HPLC analysis of the products obtained by digestion of the crude product with snake venom phosphodiesterase and bacterial alkaline phosphatase.[26] The digests of **42** provided only four kinds of nucleosides, dA:dC:dG:T = 20.4:12.1:12.5:14.9, which agrees well with the calculated ratio, 20:12:13:15, confirming the high purity of **42**; few undesired compounds, including the *N*-dimethoxytritylated, *N*-phosphorylated, and *N*-oxidized nucleosides, were detected. In addition, no product arising from depurination of deoxyadenosine and deoxyguanosine was observed.

The oligomers obtained by the present method were shown to be highly effective as primers for use in PCR amplification. For example, the reaction using **39** and **40** as universal primers Fw and Rv, respectively, and two kinds of vectors, the *Sphaeroides motA* gene[27] inserted into the pSU41 vector

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(25) Another synthesis using benzoyl protectors for adenyl and cytosyl bases and isobutyryl protectors for guanyl moieties was also examined, but the CGE of the crude product showed only broad signals and no clearly-separated peaks. This result indicated that, in this synthesis, removal of the protecting groups requiring harsh basic conditions (concentrated ammonia, 55 °C, 12 h) caused considerable decomposition of the oligomer, such as cleavage of internucleotide linkage (see ref 24).

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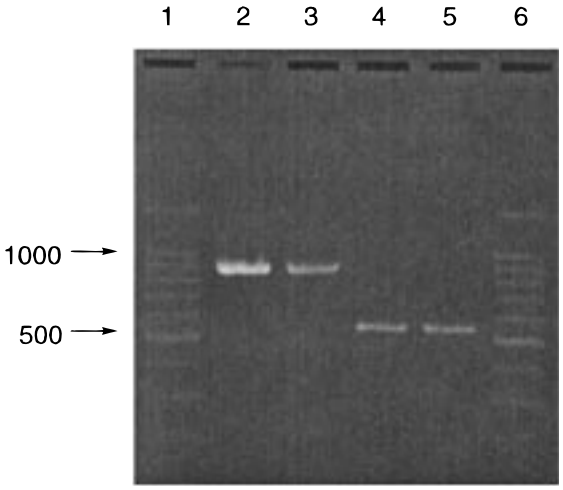

Figure 4. Etidium bromide-stained 2% agarose gel showing the size variant of the PCR-amplified plasmids of pYA701 and pSK602 with **39** and **40** (crude forms) synthesized by the N-unprotected method (lanes 2 and 4) and purchased from Pharmacia Biotech (lanes 3 and 5) as primers. Takara 100-bp DNA ladder was used as a standard DNA marker (lanes 1 and 6).

(pYA701)[28] and the pYA2032 H−E fragment inserted into the pSU41 vector (pSK602),[28] as templates was carried out on an Applied Biosystems GeneAmp PCR System Model 9700 to give the target 0.9- and 0.5-kb oligomers, respectively. Figure 4 exhibits agarose gel electrophoresis profiles of the crude products, showing that they have a high purity, which compares favorably to that of oligomers prepared with commercially supplied primers from Amersham Pharmacia Biotech.

Conclusion

We have demonstrated an efficient synthesis of oligodeoxyribonucleotides by the phosphoramidite approach without nucleoside base protection, in which key reactions include the highly chemoselective condensation of a nucleoside phosphoramidite and a nucleoside promoted by **1**, as well as the quantitative removal of a phosphityl group on the amino groups of the adenine and cytosine moieties by the benzimidazolium triflate−methanol treatment. The products generally have an excellent purity in the crude form and can be applied to a variety of molecular biological or biochemical purposes, including PCR amplification of DNAs. This new approach is superior to the existing methods in that it is easier to perform, gives higher yields of monomer units, and runs little risk of depurination of adenosine and guanosine bases. Further, this method is expected to be useful for the synthesis of DNA oligomers with base-labile modified backbones, such as alkylphosphonates, phosphotriesters, and phosphorothioates.[29]

Experimental Section

General Methods. Melting points (mp) are uncorrected. IR spectra were measured in KBr on a JASCO FT/IR-5300 spectrometer. UV spectra were taken in MeOH on a JASCO Ubest-55 spectrometer. NMR spectra were obtained in $CDCl_3$ unless otherwise noted on a JEOL α-400 instrument. The ^1H and ^{13}C NMR chemical shifts are described as δ values in parts per million relative to $(CH_3)_4Si$. ^{31}P NMR chemical shifts are reported as δ values in parts per million downfield from 85% H_3PO_4. High-performance liquid chromatography (HPLC) using a COSMOSIL 5C18-MS column (ODS-5 μm, 100 Å) was carried out on a JASCO 88-PU chromatograph with a JASCO 870-UV-absorption detector. A solid-phase syntheses were conducted on a Model 381A DNA synthesizer of Applied Biosystems. Capillary gel electrophoresis (CGE) was done in ISIS Pharmaceuticals, Inc. PCR was performed on a GeneAmp PCR System Model 9700 of Applied Biosystems. Time-of-flight (TOF) mass analysis was achieved at the Medical School, Nagoya University. Measurement of electrospray (ES) mass spectra was carried out at Tokyo Medical and Dental University. Elemental analysis was performed at the Graduate School of Human Informatics or the Faculty of Agriculture, Nagoya University. E. Merck Kieselgel 60 (70−230 mesh) deactivated by adding 6% of water was used for column chromatography. Unless otherwise stated, reactions were carried out at ambient temperature. Acetonitrile was distilled from CaH_2. Other organic solvents were used after simple distillation of the commercially supplied ones.

Materials. Imidazole (Nacalai Tesque), benzimidazole (Nacalai Tesque), trifluoromethanesulfonic acid (Central Glass), (dimethoxy)(dimethylamino)methane (Nacalai Tesque), 2′-deoxyadenosine (Yamasa), 2′-deoxycytidine hydrochloride (Yamasa), 2′-deoxyguanosine (Yamasa), thymidine (Yamasa), and 5′-*O*-(*p*,*p*′-dimethoxytrityl)thymidine 3′-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) (**9**) (Applied Biosystems, Inc.) were commercially supplied. 5′-*O*-(*p*,*p*′-Dimethoxytrityl)-2′-deoxyadenosine (**2**),[14] 5′-*O*-(*p*,*p*′-dimethoxytrityl)-2′-deoxycytidine (**3**),[16] 5′-*O*-(*p*,*p*′-dimethoxytrityl)-2′-deoxyguanosine (**4**),[7] 5′-*O*-(*p*,*p*′-dimethoxytrityl)thymidine (**5**),[17] 3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (**14**),[30] 3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxycytidine (**15**),[30] 3′-*O*-(*tert*-butyldimethylsilyl)thymidine (**16**),[30] and bis(trimethylsilyl)peroxide (TMSOOTMS)[21,31] and a toluene solution of anhydrous *tert*-butyl hydroperoxide[21,32] were prepared by the reported methods.

Imidazolium Triflate (1). Method A. Trifluoromethanesulfonic acid (6.6 g, 3.9 mL, 44 mmol) was added over 30 min to a solution of imidazole (3.0 g, 44 mmol) in dichloromethane (10 mL), and the mixture was stirred for 30 min. The reaction mixture was diluted with diethyl ether (20 mL). The occurring crystalline **1** (9.5 g, 99% yield), mp 197−198 °C, was collected by filtration. IR: 3142, 1589, 1449, 1254 cm^{-1}. ^1H NMR (CD_3OD): 8.08 (s, 2H), 9.36 (s, 1H). ^{13}C NMR (CD_3OD): 119, 122, 134. Anal. Calcd for $C_4H_5F_3N_2O_3S$: C, 22.02; H, 2.31; N, 12.84. Found: C, 21.96; H, 2.30; N, 12.74. This material was used without further purification for the condensation of a nucleoside and a nucleoside phosphoramidite.

Method B. To a solution of imidazole (3.0 g, 44 mmol) in a mixture of methanol (10 mL) and diethyl ether (10 mL) was slowly added a solution of trifluoromethanesulfonic acid (6.6 g, 3.9 mL, 44 mmol) over 10 min at 0 °C, and stirring was continued at the same temperature for additional 30 min. To the reaction mixture was added diethyl ether (30 mL), and the resulting **1** as crystals (9.5 g, 99% yield) was collected by filtration.

5′-*O*-(*p*,*p*′-Dimethoxytrityl)-2′-deoxycytidine (3). To a homogeneous mixture of 2′-deoxycytidine hydrochloride (10.5 g, 40.0 mmol), dichloroacetic acid (3.30 mL, 5.16 g, 40.0 mmol), and triethylamine (5.58 mL, 4.05 g, 40.0 mmol) in dry pyridine was added *p*,*p*′-dimethoxytrityl chloride (14.9 g, 44.4 mmol), and the resulting solution was stirred at 25 °C for 1.5 h. The reaction mixture was quenched by adding methanol (2.0 mL) and concentrated. The occurring gummy material was dissolved in dichloromethane (100 mL) and washed with an aqueous sodium hydrogencarbonate solution (40 mL) followed by brine (40 mL). Concentration of the organic layer gave an orange gum (28 g), which was treated with a mixture of acetone and toluene to afford **3** (15.4 g, 70% yield) as a colorless powder. ^1H NMR: 2.01−2.10 (m, 1H), 2.39−2.48 (m, 1H), 3.22−3.34 (m, 2H), 3.63 (s, 6H), 3.86−3.91 (m, 1H), 4.32−4.38 (m, 1H), 5.41 (d, 1H, J = 6.8 Hz), 6.10 (t, 1H, J = 5.9 Hz), 6.64−6.69 (m, 5H), 7.05−7.24 (m, 11H), 7.75 (d, 1H, J = 7.32 Hz).

5′-*O*-(*p*,*p*′-Dimethoxytrityl)-2′-deoxyguanosine (4). To a suspension of 2′-deoxyguanosine (10.7 g, 40.0 mmol) in dry methanol (100 mL) was added (dimethoxy)(dimethylamino)methane (9.66 mL, 9.53

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g, 80.0 mmol), and the mixture was stirred at 55 °C for 15 h. The reaction mixture was cooled to ambient temperature. The resulting colorless precipitates were collected by filtration and dried in vacuo to give **17** (12.4 g, 96% yield). This material (12.2 g, 37.9 mmol) and *p,p′*-dimethoxytrityl chloride (13.5 g, 40.0 mmol) and **17** (12.2 g, 37.9 mmol) were dissolved in dry pyridine (50 mL), and the resulting solution was stirred at 25 °C for 12 h. The reaction mixture was diluted with methanol (2.0 mL). Concentration of the organic solution produced a viscous oil, which was dissolved in dichloromethane (100 mL). The solution was washed with a sodium hydrogencarbonate solution (40 mL) and brine (40 mL). The organic layer was evaporated to give an orange gum (30 g). The crude material was subjected to column chromatography on silica gel with a 1:20 mixture of methanol and dichloromethane as eluent, giving **18** (16.1 g, 68% yield from **17**) as a colorless amorphous solid. The product **18** (16.0 g, 25.6 mmol) was then treated with pyridine (50 mL) containing concentrated ammonia (10 mL) at 25 °C for 24 h. The resulting solution was poured into vigorously stirred water (10 mL) to form colorless precipitates, which were collected by filtration and dried in vacuo to give **4** (14.1 g, 97% yield from **18**; 63% overall yield from 2′-deoxyguanosine) as a powder. ^{1}H NMR: 2.22−2.31 (m, 1H), 2.57−2.67 (m, 1H), 3.09−3.15 (m, 2H), 3.71 (s, 6H), 3.87−3.91 (m, 1H), 4.32−4.35 (m, 1H), 5.28 (d, 1H, J = 4.4 Hz), 6.12 (dd, 1H, J = 6.3, 6.8 Hz), 6.42 (br s, 2H), 6.81 (dd, 4H, J = 6.8, 8.8 Hz), 7.17−7.34 (m, 9H), 7.76 (s, 1H), 10.58 (br s, 1H). This material was used for the next preparation of the 3′-phosphoramidite without further purification.

5′-*O*-(*p,p′*-Dimethoxytrityl)-2′-deoxyadenosine 3′-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite) (6). A Typical Procedure for the Preparation of N-Unprotected Nucleoside 3′-Phosphoramidites. Method A. A solution of 5′-*O*-(*p,p′*-dimethoxytrityl)-2′-deoxyadenosine (**2**) (11.1 g, 20.0 mmol) and ethyldiisopropylamine (3.88 g, 5.23 mL, 30.0 mmol) in tetrahydrofuran (40 mL) was cooled to −78 °C, and to this was added (2-cyanoethyl)(*N,N*-diisopropylamino)chlorophosphine (4.97 g, 4.68 mL, 21.0 mmol). The mixture was warmed to 25 °C and stirred at the same temperature for 60 min. The reaction mixture was washed with an aqueous sodium hydrogencarbonate solution (100 mL) and brine (100 mL). The organic solution was dried over Mg_2SO_4 and concentrated to give a viscous oil, which was dissolved in dichloromethane (50 mL). The resulting solution was poured into a vigorously stirred petroleum ether (2.0 L) to precipitate a colorless powder. Filtration of the solid material gave **6** (14.7 g 97% yield). IR: 1757, 1612, 1510, 1361 cm^{-1}. UV: λ_{max} 237 (ϵ 22 200), 267 nm (16 800). ^{1}H NMR: 1.05−1.33 (m, 12H), 2.42 (t, 1H, J = 10.5 Hz), 2.43−2.72 (m, 2H), 2.81−2.93 (m, 1H), 3.22−3.78 (m, 12H), 4.23−4.55 (m, 1H), 4.70−4.83 (m, 1H), 6.04−6.20 (br s, 2H), 6.41−6.49 (m, 1H), 6.72−6.78 (m, 4H), 7.13−7.42 (m, 9H), 7.96, 8.01 (2 s, 1H), 8.25 (s, 1H). ^{31}P NMR: 149.4, 149.3. Anal. Calcd for $C_{40}H_{48}N_7O_6P$: C, 63.73; H, 6.42; N, 13.01. Found: C, 63.70; H, 6.56; N, 13.01. This product was employed without further purification to the solid-phase synthesis of oligonucleotides.

Method B. To a suspension of 5′-*O*-(*p,p′*-dimethoxytrityl)-2′-deoxyadenosine (**2**) (11.1 g, 20.0 mmol) and freshly distilled (2-cyanoethyl)bis(diisopropylamino)phosphine (6.63 g, 6.99 mL, 22.0 mmol) in dichloromethane (40 mL) was added **1** (1.31 g, 6.00 mmol) in three portions. The resulting mixture was stirred for 60 min. The reaction mixture was diluted with dichloromethane (100 mL) and washed with an aqueous solution of sodium hydrogencarbonate (100 mL) and brine (100 mL). The organic layer was dried over Mg_2SO_4 and concentrated to ca. 50 mL of the volume. The resulting solution was added to a vigorously stirred petroleum ether (2.0 L) to afford **6** (14.5 g, 96% yield) as colorless precipitates.

5′-*O*-(*p,p′*-Dimethoxytrityl)-2′-deoxycytidine 3′-(2-Cyanoethyl *N,N*-diisopropyl-phosphoramidite) (7). Compound **7** was obtained as a colorless powder. IR: 1759, 1610, 1511, 1362 cm^{-1}. UV: λ_{max} 236 (ϵ 19 200), 267 nm (16 000). ^{1}H NMR: 0.95−1.09 (m, 12H), 2.03−2.19 (m, 1H), 2.25 (t, 1H, J = 10.4 Hz), 2.42−2.55 (m, 2H), 3.22−3.56 (m, 5H), 3.57−3.72 (m, 7H), 4.03−4.09 (m, 1H), 4.41−4.55 (m, 1H), 5.40−5.55 (m, 1H), 6.10−6.27 (m, 1H), 6.70−6.81 (m, 4H), 7.08−7.36 (m, 11H), 7.67, 7.78 (2 s, 1H). ^{31}P NMR: 148.7, 148.3. Anal. Calcd for $C_{39}H_{48}N_5O_7P$: C, 64.18; H, 6.63; N, 9.60. Found: C, 63.99; H, 6.62; N, 9.88.

5′-*O*-(*p,p′*-Dimethoxytrityl)-2′-deoxyguanosine 3′-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite) (8). Compound **8** was obtained as a colorless powder. IR: 1762, 1611, 1523, 1365 cm^{-1}. UV: λ_{max} 237 (ϵ 22 100), 268 nm (17 200). ^{1}H NMR 1.01−1.25 (m, 12H), 2.41 (t, 1H, J = 10.5 Hz), 2.43−2.77 (m, 3H), 3.31−3.80 (m, 12H), 4.11−4.18 (m, 1H), 4.55−4.61 (m, 1H), 6.43−6.49 (m, 1H), 6.74−6.80 (m, 4H), 7.10−7.36 (m, 11H), 7.38−7.43 (m, 1H), 7.60−7.69 (m, 1H). ^{31}P NMR: 149.4, 149.3. Anal. Calcd for $C_{40}H_{48}N_7O_7P$: C, 62.41; H, 6.28; N, 12.74. Found: C, 62.40; H, 6.39; N, 12.55.

Preparation of 2-Cyanoethyl [5′-*O*-(*p,p′*-Dimethoxytrityl)-2′-deoxyadenylyl]-(3′-5′)-3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (19). A Typical Procedure for the Synthesis of Dinucleoside Phosphates Using Stoichiometric Amounts of 1, an N-Free Nucleoside 3′-Phosphoramidite, and an N-Free Nucleoside in a Solution Phase. A solution of **1** (43.6 mg, 0.20 mmol), the phosphoramidite **6** (302 mg, 0.40 mmol), and the nucleoside **14** (146 mg, 0.40 mmol) in dry acetonitrile (0.8 mL) was stirred at room temperature for 1 min. To this mixture was added a 1.0 M toluene solution of *tert*-butyl hydroperoxide (TBHP) (0.60 mL, 0.60 mmol), and stirring was continued for 5 min. Evaporation of the reaction mixture gave an oil, which was dissolved in a 1:10 mixture of methanol and dichloromethane (2.0 mL). The solution was passed through a silica gel pad (2.00 g) and washed with a 1:10 mixture of methanol and dichloromethane (5.0 mL). The organic filtrate was concentrated to afford **19** (393 mg, 95% yield) as a colorless amorphous solid. IR: 1735, 1686, 1654, 1648, 1611, 1508, 1466 cm^{-1}. UV: λ_{max} 238 (ϵ 30700), 260 nm (34100); ^{1}H NMR 0.06 (s, 3H), 0.07 (s, 3H), 1.18 (s, 9H), 2.45−2.80 (m, 3H), 2.95−3.05 (m, 2H), 3.26−3.55 (m, 3H), 3.74, 3.75 (2 s, 3H), 3.88−3.93 (d, 1H, J = 12.5 Hz), 4.01−4.42 (m, 4H), 4.65 (d, 2H, J = 4.6 Hz), 5.15−5.24 (m, 1H), 6.21−6.26 (m, 1H), 6.43−6.57 (m, 4H), 6.74−6.85 (m, 4H), 7.12−7.37 (m, 10H), 7.82−8.02 (m, 2H), 8.17 (m, 2H). ^{31}P NMR: −2.2, −2.0. Anal. Calcd for $C_{50}H_{60}N_{11}O_{10}PSi$: C, 58.07; H, 5.85; N, 14.90. Found: C, 58.03; H, 5.77; N, 14.84.

2-Cyanoethyl [5′-*O*-(*p,p′*-Dimethoxytrityl)-2′-deoxyadenylyl]-(3′-5′)-3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxycytidine (20). Compound **20** was obtained as a colorless amorphous solid (yield 93%). IR: 1718, 1686, 1654, 1608, 1509, 1370 cm^{-1}. UV: λ_{max} 232 (ϵ 60 100), 260 nm (23 100). ^{1}H NMR: 0.07 (s, 3H), 0.10 (s, 3H), 1.12 (s, 9H), 2.06−2.40 (m, 2H), 2.51−3.03 (m, 4H), 3.35−3.46 (m, 4H), 3.77, 3.78 (2 s, 3H), 3.84−4.48 (m, 7H), 5.17−5.29 (m, 1H), 5.83−5.98 (m, 2H), 6.44−6.47 (m, 2H), 6.77−6.80 (m, 4H), 7.19−7.38 (m, 10H), 7.84−8.06 (m, 2H), 8.26 (d, 1H, J = 5.9 Hz). ^{31}P NMR: −2.0, −1.9. Anal. Calcd for $C_{49}H_{60}N_9O_{11}PSi$: C, 58.26; H, 5.99; N, 12.48. Found: C, 57.99; H, 6.08; N, 12.44.

2-Cyanoethyl [5′-*O*-(*p,p′*-Dimethoxytrityl)-2′-deoxyadenylyl]-(3′-5′)-3′-*O*-(*tert*-butyldimethylsilyl)thymidine (21). Compound **21** was obtained as a colorless amorphous solid (yield 95%). IR: 1701, 1607, 1560, 1508, 1474, 1406 cm^{-1}. UV λ_{max} 236 (ϵ 28 700), 261 nm (26 100). ^{1}H NMR 0.05 (s, 3H), 0.08 (s, 3H), 1.23, 1.24 (2 s, 9H), 2.01−2.08 (m, 1H), 2.18−2.33 (m, 2H), 2.72−2.76 (m, 3H), 3.35−3.51 (m, 4H), 3.75 (s, 6H), 3.81−4.49 (m, 7H), 4.71 (d, 2H, J = 4.6 Hz), 5.15−5.28 (m, 1H), 6.14−6.43 (m, 3H), 6.76−6.78 (m, 4H), 7.16−7.37 (m, 9H), 7.45 (s, 1H), 7.60 (s, 1H), 8.04−8.10 (m, 1H), 8.29−8.31 (m, 1H), 8.84−9.89 (br s, 1H). ^{31}P NMR: −2.1, −2.0. Anal. Calcd for $C_{50}H_{61}N_8O_{12}PSi$: C, 58.58; H, 6.00; N, 10.93. Found: C, 58.33; H, 5.83; N, 11.05.

2-Cyanoethyl [5′-*O*-(*p,p′*-Dimethoxytrityl)-2′-deoxycytosylyl]-(3′-5′)-3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (22). Compound **22** was obtained as a colorless amorphous solid (yield 96%). IR: 1701, 1607, 1508, 1474 cm^{-1}. UV: λ_{max} 239 (ϵ 39 400), 261 nm (51 800). ^{1}H NMR 0.07 (s, 3H), 0.12 (s, 3H), 1.38 (s, 9H), 2.18−2.74 (m, 6H), 3.30−3.55 (m, 4H), 3.78, 3.79 (2 s, 6H), 3.86−4.19 (m, 7H), 4.69−4.75 (m, 1H), 5.07−5.11 (m, 1H), 6.28−6.67 (m, 4H), 6.81−6.84 (m, 4H), 7.11−7.39 (m, 9H), 7.48−7.62 (m, 1H), 7.98 (m, 1H), 8.33 (s, 1H). ^{31}P NMR: −2.2. Anal. Calcd for $C_{49}H_{60}N_9O_{11}PSi$: C, 58.26; H, 5.99; N, 12.48. Found: C, 58.26; H, 5.97; N, 12.56.

2-Cyanoethyl [5′-*O*-(*p,p′*-Dimethoxytrityl)-2′-deoxycytosylyl]-(3′-5′)-3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxycytidine (23). Compound **23** was obtained as a colorless amorphous solid (yield 95%). IR: 1749, 1671, 1613, 1584, 1508, 1464 cm^{-1}. UV: λ_{max} 235 (ϵ 44 800), 269 nm (29 000). ^{1}H NMR 0.05 (s, 3H), 0.12 (s, 3H), 1.34, 1.36 (2 s, 9H),

2.02−2.38 (m, 3H), 2.52−2.81 (m, 3H), 3.32−3.46 (m, 4H), 3.80−4.46 (m, 13H), 5.00−5.13 (m, 1H), 5.59−5.72 (m, 1H), 5.87 (d, 1H, J = 7.8 Hz), 6.10−6.43 (m, 3H), 6.83 (d, 4H, J = 8.3 Hz), 7.10−7.37 (m, 10H), 7.53−7.94 (m, 1H), 7.99 (d, 1H, J = 7.8 Hz). ^{31}P NMR: −1.9, −1.6. Anal. Calcd for $C_{48}H_{60}N_7O_{12}PSi$: C, 58.47; H, 6.13; N, 9.94. Found: C, 58.57; H, 6.24; N, 9.84.

2-Cyanoethyl [5′-*O*-(*p,p*′-Dimethoxytrityl)-2′-deoxycytosylyl]-(3′-5′)-3′-*O*-(*tert*-butyldimethylsilyl)thymidine (24). Compound **24** was obtained as a colorless amorphous solid (yield 94%). IR: 1701, 1507, 1474 cm^{-1}. UV: λ_{max} 235 (ϵ 42 800), 271 nm (23 400). ^{1}H NMR: 0.07 (s, 3H), 0.08 (s, 3H), 1.37 (s, 9H), 2.01−2.07 (m, 1H), 2.17−2.21 (m, 3H), 2.58−2.79 (m, 2H), 3.39−3.45 (m, 2H), 3.68−3.93 (m, 13H), 4.26−4.49 (m, 4H), 5.01−5.12 (m, 1H), 6.15−6.34 (m, 3H), 6.83−6.85 (m, 4H), 7.23−7.33 (m, 9H), 7.46 (s, 1H), 7.71 (d, 1H, J = 7.3 Hz), 9.10−9.55 (br s, 1H). ^{31}P NMR −2.2, −1.9. Anal. Calcd for $C_{49}H_{61}N_6O_{13}PSi$: C, 58.79; H, 6.14; N, 8.39. Found: C, 58.67; H, 5.98; N, 8.45.

2-Cyanoethyl [5′-*O*-(*p,p*′-Dimethoxytrityl)-2′-deoxyguanylyl]-(3′-5′)-3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (25). Compound **25** was obtained as a colorless amorphous solid (yield 96%). IR: 1749, 1671, 1613, 1584, 1508, 1464 cm^{-1}. UV λ_{max} 238 (ϵ 38 800), 256 nm (36 000). ^{1}H NMR: 0.11 (s, 3H), 0.13 (s, 3H), 1.33, 1.34 (2 s, 9H), 2.18−2.22 (m, 1H), 2.41−2.79 (m, 3H), 2.84−3.03 (m, 2H), 3.37−3.84 (m, 4H), 3.93−4.29 (m, 13H), 5.16−5.28 (m, 1H), 6.10−6.47 (m, 3H), 6.74−6.82 (m, 4H), 7.14−7.37 (m, 10H), 7.62−7.71 (m, 1H), 7.87 (s, 1H), 8.19 (s, 1H), 11.59−11.86 (br s, 1H). ^{31}P NMR: −2.5, −2.2. Anal. Calcd for $C_{50}H_{60}N_{11}O_{11}PSi$: C, 57.19; H, 5.76; N, 14.67. Found: C, 57.23; H, 5.84; N, 14.59.

2-Cyanoethyl [5′-*O*-(*p,p*′-Dimethoxytrityl)-2′-deoxyguanylyl]-(3′-5′)-3′-*O*-(*tert*-butyldimethylsilyl)thymidine (26). Compound **26** was obtained as a colorless amorphous solid (yield 98%). IR: 1691, 1609, 1535, 1508, 1473 cm^{-1}. UV: λ_{max} 238 (ϵ 43 900), 268 nm (30 800). ^{1}H NMR 0.05 (s, 3H), 0.06 (s, 3H), 1.31, 1.33 (2 s, 9H), 2.02−2.07 (m, 3H), 2.54−2.75 (m, 3H), 2.72−2.76 (m, 3H), 3.35−3.40 (m, 4H), 3.69 (s, 6H), 3.74−4.47 (m, 7H), 5.23−5.28 (m, 1H), 6.15−6.34 (m, 3H), 6.76−6.78 (m, 4H), 7.16−7.36 (m, 9H), 7.50 (s, 1H), 7.60 (s, 1H), 11.25−11.93 (br s, 1H). ^{31}P NMR: −2.2, −2.0. Anal. Calcd for $C_{50}H_{61}N_8O_{13}PSi$: C, 57.68; H, 5.91; N, 10.76. Found: C, 57.66; H, 5.82; N, 11.00.

2-Cyanoethyl [5′-*O*-(*p,p*′-Dimethoxytrityl)thymylyl]-(3′-5′)-3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (27). Compound **27** was obtained as a colorless amorphous solid (yield 96%). IR: 1701, 1681, 1608, 1474 cm^{-1}. UV: λ_{max} 273 (ϵ 19 500), 261 nm (20 300). ^{1}H NMR 0.08 (s, 3H), 0.09 (s, 3H), 1.34, 1.36 (2 s, 9H), 2.17−2.20 (m, 1H), 2.20−2.78 (m, 5H), 3.25−3.57 (m, 4H), 3.76, 3.77 (2 s, 6H), 3.85−4.32 (m, 7H), 5.10−5.12 (m, 1H), 6.26−6.48 (m, 4H), 6.60−6.67 (br s, 2H), 6.80−6.83 (m, 4H), 7.15−7.39 (m, 12H), 8.00 (s, 1H), 8.31 (s, 1H). ^{31}P NMR: −2.3, −2.2. Anal. Calcd for $C_{50}H_{61}N_8O_{12}PSi$: C, 58.58; H, 6.00; N, 10.93. Found: C, 58.57; H, 5.96; N, 10.94.

Preparation of 2′-Deoxyadenylyl-(3′-5′)-thymidine (35) via Phosphitylation of the Solid-Anchored Thymidine 30 Using Excess Amounts of 1 and 6. To **30** (50.0 mg, 10 μmol) was added a solution of **1** (54.5 mg, 250 μmol) in acetonitrile (2.5 mL) and a solution of **6** (189 mg, 250 μmol) in acetonitrile (2.5 mL). After 1 min, an aliquot of the resins was taken up form the reaction mixture, washed with acetonitrile, and subjected to the ^{31}P NMR analysis. The NMR spectrum showed a ca. 95:5 ratio of two kinds of peaks at δ 140−141 ppm arising from phosphites and 127−128 ppm due to phosphoramidites. To the reaction mixture was added a solution of benzimidazolium triflate (268 mg, 1.0 mmol) in methanol (2.0 mL), and the mixture was stirred for 2 min. An aliquot of the resins was again subjected to the ^{31}P NMR assay. In the spectrum, only the signals at δ 140−141 ppm due to the phosphites were observed. The reaction mixture was then treated with a 1.0 M solution of *tert*-butyl hydroperoxide in toluene (0.5 mL, 500 μmol) for 2 min and washed with acetonitrile (1.0 mL). The resulting resins were stirred with a 5% solution of dichloroacetic acid in dichloromethane (4 mL) for 4 min. The solution phase was removed by filtration. The resulting solid material was washed with acetonitrile and exposed to concd ammonia (4.0 mL) for 180 min. The aqueous layer was collected and concentrated to give an oil, which was treated with ethanol to afford the ammonium salt of **35** (5.25 mg, 94% yield) as an oil.

Preparation of 2′-Deoxycytosylyl-(3′-5′)-thymidine (36) via Phosphitylation of 30 Using Excess Amounts of 1 and 7. In a manner similar to that described above, the ammonium salt of **36** (5.15 mg, 96% yield) was prepared using **7** (183 mg, 250 μmol), **30** (50.0 mg, 10 μmol), and **1** (54.5 mg, 250 μmol).

Preparation of 2′-Deoxyguanylyl-(3′-5′)-thymidine (37) via Phosphitylation of the Solid-Anchored Thymidine 30 Using Excess Amounts of 1 and 8. To **30** (50.0 mg, 10 μmol) was added a solution of **1** (54.5 mg, 250 μmol) in acetonitrile (2.5 mL) and a solution of **8** (193 mg, 250 μmol) in acetonitrile (2.5 mL). After 1 min, an aliquot of the resins was subjected to the ^{31}P NMR spectrum, which showed only signals due to phosphites at δ 140−141 ppm. To the reaction mixture was added a 1.0 M solution of *tert*-butyl hydroperoxide in toluene (0.5 mL, 500 μmol), and stirring was continued for 2 min. The reaction mixture was filtered, and the resulting resins were exposed to a 5% dichloromethane solution of dichloroacetic acid (4.0 mL) for 4 min. The organic layer was filtered off, and the solid was treated with concentrated ammonia (2.0 mL) for 180 min. Concentration of the aqueous layer afforded the ammonium salt of **37** (5.40 mg, 94% yield) as an oil.

Preparation of Thymylyl-(3′-5′)-thymidine (38) via Phosphitylation of 30 Using Excess Amounts of 1 and 9. According to a procedure similar to that for preparation of **37**, the ammonium salt of **38** (5.20 mg, 95% yield) was obtained using **30** (50.0 mg, 10 μmol), **1** (55.0 mg, 250 μmol), and **9** (186 mg, 250 μmol).

HPLC Analysis of the Enzymatic Digestion Products. A mixture of the purified oligoDNA (0.2−1.5 OD_{260}) in H_2O (31.5 mL) synthesized by the N-unprotected method, snake venom phosphodiesterase (SVP) (1 mL, 0.1 unit), and bacterial alkaline phosphatase (BAP) (10 mL, 2.5 unit) in 300 mM Tris-HCl (5 mL) and 300 mM $MgCl_2$ (2.5 mL) was incubated at 37 °C for 12 h and then heated at 90 °C for 0.5 min. The aliquots (5 mL) were injected directly onto an COSMOSIL 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 = 3.5 min; dG, 4.6 min; T, 5.8 min; dA, 7.0 min: eluted with H_2O−MeOH (9:1)]. **41**: Found: dC, 7.3; dG, 2.8; T, 9.0; dA, 12.7. Calcd: dC, 7; dG, 3; T, 9; dA, 13. **42**: Found: dC, 12.1; dG, 12.5; T, 14.9; dA, 20.4. Calcd: dC, 12; dG, 13; T, 15; dA, 20.

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Supporting Information Available: Characterization data including ^{1}H and ^{31}P NMR spectral charts for all new compounds (27 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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