Preparation of Short Oligonucleotides via the Phosphoramidite Method Using a Tetrazole Promoter in a Catalytic Manner

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Abstract: A facile phosphoramidite method using a tetrazole promoter in a catalytic manner has been developed for the condensation of a nucleoside 3′-phosphoramidite and a nucleoside. This method is particularly useful for a large-scale synthesis of short oligonucleotides. For example, dinucleoside phosphates are prepared on a multigram scale in 92−99% yields through the reaction of nucleoside 3′-*N*,*N*-diethylphosphoramidites (1.05 equiv) and 5′-O-free nucleosides (1.00 equiv) with 5-(*p*-nitrophenyl)-1*H*-tetrazole (NPT) (0.05 equiv) in the presence of molecular sieves 13X in acetonitrile (40 °C, 60 min) followed by trimethylsilyl triflate-catalyzed oxidation with bis(trimethylsilyl) peroxide in dichloromethane (40 °C, 10 min). The NPT-catalytic approach is also effective for the synthesis of longer deoxyribonucleotides such as d($^{5'}$CTACCTGT$^{3'}$) and 2′−5′- or 3′−5′-linked ribonucleotides.

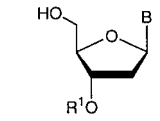
Introduction

An efficient large-scale synthesis of short oligonucleotides in a solution phase has become an important subject in nucleic acid chemistry. The synthesis is currently achieved by condensation of a nucleoside and a nucleoside phosphoramidite with promotion by a tetrazole such as 1*H*-tetrazole,[1] 5-(*p*-nitrophenyl)-1*H*-tetrazole (NPT),[2] or 5-(ethylthio)-1*H*-tetrazole[3] as a key step.[4] This reaction usually requires 2−4 equiv each of a promoter and a phosphoramidite to a nucleoside for gaining an acceptable reaction rate.[5] However, the use of an excess of the tetrazole compound, particularly NPT, poses serious problems in large-scale synthesis because they are fairly expensive, harmful to health,[6] potentially explosive, and poorly soluble in a reaction solvent. Thus, methods using a *catalytic* amount of the tetrazole activator have been strongly desired, but not yet viable.[5b,c,7] This paper describes the first tetrazole-catalyzed condensation of a nucleoside and a nucleoside phosphoramidite.

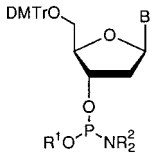
Results and Discussion

In the phosphoramidite method, the acidic tetrazole acts not only as an activator of the amidite but also as a scavenger of the generated amine, where the scavenger takes precedence over the activator because the amine is a stronger base than the phosphoramidite. Hence, a stoichiometric amount of the tetrazole is the minimum required for completion of the reaction. Here, if the generated amine can be removed, the tetrazole would catalytically promote the condensation as shown in Scheme 1. This was actually achieved by the use of molecular sieves (MS) 13X (10 Å pore size; ca. 2 μm particle size) as the amine scavenger.

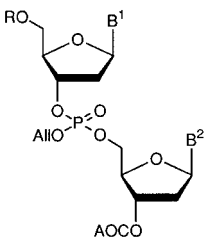
The use of an *O*-allyl *N*,*N*-diethylphosphoramidite as the amidite, NPT as the promoter, and MS 13X as the amine scavenger is most effective for the reaction to proceed at a reasonable rate and with a high yield. For example, the condensation of the nucleoside **2** (1.00 equiv) and the amidite



1, B = Cy^{AOC}; R^1 = AOC
2, B = Th; R^1 = AOC
3, B = Th; R^1 = TBDMS



4, B = Ad^{AOC}; R^1 = CH_2=$CHCH_2$; R^2 = Et
5, B = Cy^{AOC}; R^1 = CH_2=$CHCH_2$; R^2 = Et
6, B = $Gu^{All,AOC}$; R^1 = CH_2=$CHCH_2$; R^2 = Et
7, B = Th; R^1 = CH_2=$CHCH_2$; R^2 = Et
8, B = Th; R^1 = CH_2=$CHCH_2$; R^2 = Me
9, B = Th; R^1 = $CNCH_2CH_2$; R^2 = Et
10, B = Th; R^1 = *o*-ClC_6H_4; R^2 = Et



11, B^1 = Ad^{AOC}; B^2 = Cy^{AOC}; R = DMTr
12, B^1 = B^2 = Cy^{AOC}; R = DMTr
13, B^1 = $Gu^{All,AOC}$; B^2 = Cy^{AOC}; R = DMTr
14, B^1 = Th; B^2 = Cy^{AOC}; R = DMTr
15, B^1 = Ad^{AOC}; B^2 = Th; R = DMTr
16, B^1 = Cy^{AOC}; B^2 = Th; R = DMTr
17, B^1 = $Gu^{All,AOC}$; B^2 = Th; R = DMTr
18, B^1 = B^2 = Th; R = DMTr
19, B^1 = $Gu^{All,AOC}$; B^2 = Th; R = H

[⊗] Abstract published in *Advance ACS Abstracts,* November 15, 1997.

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(6) *Sigma-Aldrich Library of Chemical Safety 2*; Sigma-Aldrich: Milwaukee, 1990; p 3313D (Sigma-Aldrich Material Safety Data Sheets; Product No. 33644-0).
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Scheme 1

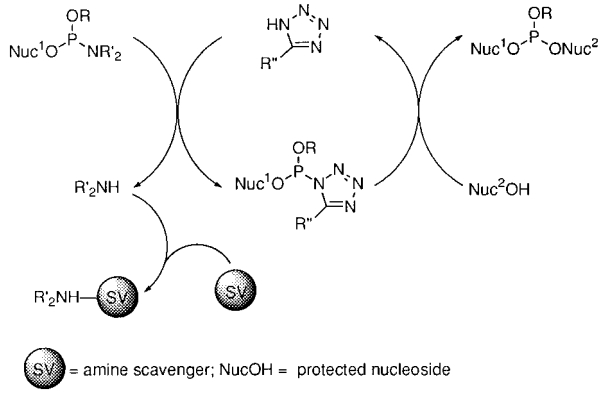

Table 1. Synthesis of Dinucleoside Phosphates[a]

nucleoside	amidite	product	yield,[b] %
1	**4**	**11**	97
1	**5**	**12**	96
1	**6**	**13**	92
2	**7**	**14**	98
2	**4**	**15**	97
2	**5**	**16**	97
2	**6**	**17**	95
2	**7**	**18**	99

[a] Condensation was carried out using amidite, nucleoside, and NPT in a 1.05:1.00:0.05 molar ratio in acetonitrile at 40 °C for 60 min. The resulting phosphite was directly oxidized by bis(trimethylsilyl) peroxide/trimethylsilyl triflate in acetonitrile−CH_2Cl_2 (40 °C, 5 min) to the phosphate. [b] Isolated yield. The product was obtained as a ca. 1:1 mixture of diastereomers.

7[8] (1.05 equiv) using a catalytic amount of NPT (0.05 equiv) in the presence of MS 13X in acetonitrile (40 °C, 60 min)[9] followed by oxidation with bis(trimethylsilyl) peroxide (TMSOOTMS) (2.0 equiv) assisted by trimethylsilyl triflate (TMSOTf) (0.05 equiv) in dichloromethane[10] (40 °C, 10 min) afforded **18** as a ca. 1:1 mixture of diastereomers (^{31}P NMR: −1.4 and −1.1 ppm, H_3PO_4 standard) in 99% yield. The phosphitylation was also achieved at 25 °C but required longer time (80−120 min). In this reaction, the dimethoxytrityl, (allyloxy)carbonyl (AOC), and allyl protectors were left intact. MS 13X is recyclable as the amine scavenger after drying at 200 °C for 12 h. Table 1 shows several examples of the dinucleoside phosphotriesters obtained by the new method. The products could be converted generally in >95% yield to the unprotected derivatives through detritylation with dichloroacetic acid and removal of the allyl and AOC protectors with $Pd[P(C_6H_5)_3]_4/P(C_6H_5)_3$[8,11] in the presence of diethylammonium hydrogen carbonate.[11]

We investigated the efficiency of other amine scavengers and promoters in the reaction of **2** and **7**. MS 3A and 4A with smaller pore sizes were not suitable as the amine scavenger. A weak cation exchanger such as DIAION WK-40 (H-form) was less effective, requiring 72 h longer for completion of the condensation. Mineral acids such as *p*-toluenesulfonic acid or strong acid resins such as Dowex 50W-8X and Nafion brought about decomposition of the phosphoramidite, detritylation, and, in the cases with deoxyadenosine and deoxyguanosine derivatives, depurination. The use of 1*H*-tetrazole, 5-(ethylthio)-1*H*-tetrazole, benzimidazolium triflate,[12] pyridinium chloride,[13] or *N*-methylanilinium trifluoroacetate[14] as a catalytic promoter gave little of the coupling products. Figure 1 shows some of these results including that obtained by the use of MS 13X and NPT. The utility of several phosphoramidites was also examined. Allyl *N*,*N*-dimethylphosphoramidites serve as alternative phosphoramidites. The NPT-catalyzed reaction of **2** and the freshly prepared **8** was finished in 15 min to afford after the TMSOOTMS oxidation **18** in 93% yield. The dimethylphosphoramidites, however, are not absolutely useful because they lack stability and therefore require strict conditions for storage.[15] Allyl *N*,*N*-diisopropylphosphoramidites may be used, but these substrates require longer reaction time or harder reaction conditions. That is, the condensation was generally accomplished in 24 h at 25 °C or in 1 h at reflux temperature. When less reactive allyl phosphoromorpholidites were used, the reaction was incomplete even after 12 h at reflux temperature. Amidites with the electron-withdrawing alkyl substituent such as 2-cyanoethyl or *o*-chlorophenyl in place of the allyl on the ester part decelerate the reaction. For instance, the condensation of **3** and the 2-cyanoethylated *N*,*N*-diethylphosphoramidite **9** with a catalytic amount of NPT in acetonitrile at 40 °C needed ca. 8 h for completion. The reaction using the *O*-*o*-chlorophenyl analog **10** was not finished even after 12 h; the yield of the desired product was ca. 20%.

This NPT-catalyzed approach is also useful for the preparation of longer deoxyribonucleotides. Synthesis of the octamer d(5′-

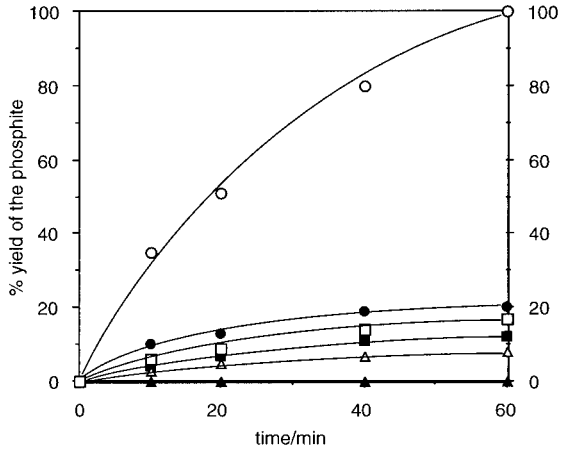

Figure 1. Profile of the condensation of **2** and **7**. The reaction was conducted using 5 mol % of a promoter in the presence or absence of an amine scavenger in acetonitrile at 40 °C. The yield of the phosphite was estimated by the ^{31}P NMR analysis with triphenylphosphine oxide as a standard; ○, NPT/MS 13X; ●, NPT/DIAION WK-40; □, benzimidazolium triflate/MS 13X; ■, 1*H*-tetrazole/MS 13X, △, NPT/without scavenger; ▲, NPT/DOWEX 50W-X8.

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(9) The controlled experiment without NPT at 40 °C did not give **18** even after 5 h. This result indicates that NPT serves as a catalyst in the reaction.

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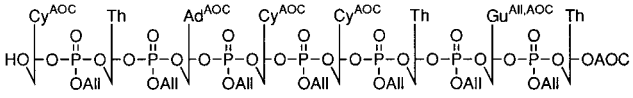
(15) In the storage of **8** under argon at −40 or +25 °C for 24 h, ca. 2 or 15% of decomposition, respectively, was observed. Cf. Sinha, N. D.; Biernat, J.; McManus, J.; Köster, H. *Nucleic Acids Res.* **1984**, *12*, 4539−4557.

CTACCTGT$^{3'}$) (**21**) was representatively demonstrated. The



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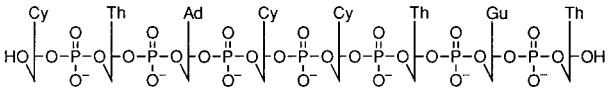

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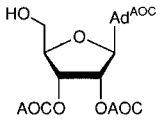

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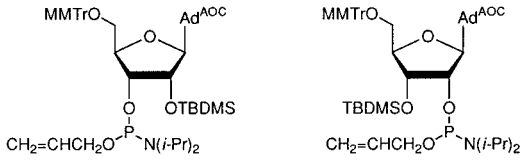

23 **24**

condensation of **2** and **6** with NPT (40 °C, 60 min) and the subsequent TMSOOTMS/TMSOTf oxidation (40 °C, 10 min) gave a diasteromeric mixture of the dinucleoside phosphate **17**, which was detritylated by dichloroacetic acid in dichloromethane (25 °C, 10 min) to afford **19** in 95% overall yield. Repetition of (1) the phosphitylation with a suitable nucleoside 3′-phosphoramidite among **4**−**7** (40 °C, 1−2 h), (2) the TMSOOTMS oxidation, and (3) the detritylation converted the dimer **19** to the protected octamer **20** in 57% overall yield.[16] In this preparation, the phosphitylation proceeded with 91−95% yield. The yield is comparable with that in the existing typical approach using 2 equiv each of the phosphoramidite and NPT. The allyl and AOC protectors of **20** were removed by treatment with catalytic amounts of $Pd[P(C_6H_5)_3]_4$ and $P(C_6H_5)_3$ in the presence of diethylammonium hydrogen carbonate in aqueous tetrahydrofuran to give **21** in ca. 91% yield. The HPLC and ^{31}P NMR analysis (Figure 2) indicated that the purity of crude **21** is >98%. HPLC of the product obtained by digestion of **21** with snake venom phosphodiesterase and bacterial alkaline phosphatase[17] showed four peaks due to dA, dC, dG, and T in a ratio of 0.9:3.0:0.9:3.1. The experimentally derived base composition agreed well with the ratio calculated for **21** to confirm the structure.

The catalytic method can also be applied to the synthesis of ribonucleotides bearing 2′−5′- or 3′−5′-internucleotide linkages. In these cases, since high quality of *N*,*N*-diethylphosphoramidites is not easily accessible, *N*,*N*-diisopropylphosphoramidites were employed. Thus, the NPT-catalyzed reaction of the nucleoside **22** and the phosphoramidite **23** in the presence of MS 13X (8 h, acetonitrile reflux temperature) followed by the TMSOOTMS/TMSOTf oxidation gave **25** in 95% yield. Similarly, the 2′−5′-linked nucleotide **26** was produced in 96% yield from **22** and **24**.

(16) Preparation of nonamers from **20** was attempted under several conditions, but it was not achieved in a satisfactory yield since the solubility of the 5′-O-free derivative of **20** in acetonitrile is too low to allow homogeneous reaction of this component and a phosphoramidite.

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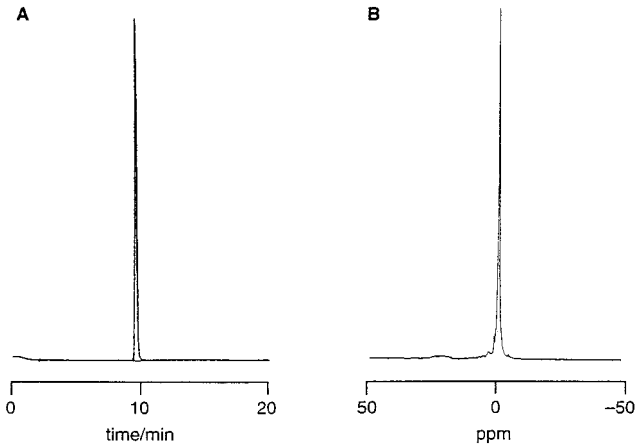
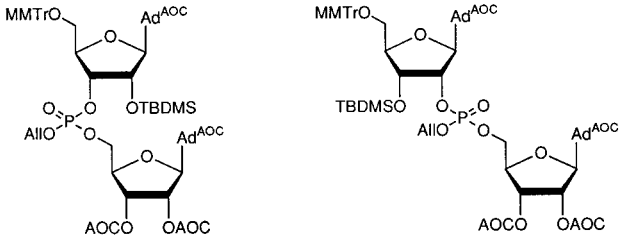
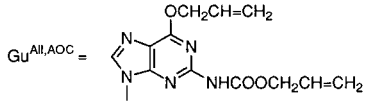


Figure 2. HPLC and ^{31}P NMR profile of the crude octamer d(5′-CTACCTGT3′) (**21**): (A) the HPLC chart obtained using an ODS-5μm column (4.6 × 250 mm) eluted with a 0.1 M triethylammonium acetate buffer containing 5−15% acetonitrile (v/v) (linear gradient in 30 min); (b) the ^{31}P NMR spectrum in D_2O with 85% H_3PO_4 as an external standard.



25 **26**

All = CH_2=$CHCH_2$; AOC = CH_2=$CHCH_2OCO$; DMTr = $C_6H_5(p\text{-}CH_3OC_6H_4)_2C$
MMTr = $p\text{-}CH_3OC_6H_4(C_6H_5)_2C$



Conclusion

We have realized for the first time a facile method for the preparation of nucleotides via the tetrazole-catalyzed condensation of a nucleoside and a nucleoside phosphoramidite using a trapping agent of the amine generated in the reaction, where the use of NPT as the promoter, the allyl *N*,*N*-diethylphosphoramidite in deoxyribonucleotide synthesis or the allyl *N*,*N*-diisopropylphosphoramidite in ribonucleotide synthesis as the phosphoramidite, and MS 13X as the amine scavenger is most effective. In the new approach, another remarkable result was obtained; that is, the condensation can be performed with an excellent yield by use of nearly equimolar amounts of a nucleoside phosphoramidite and a nucleoside. The use of the expensive promoter or nucleoside phosphoramidites in a catalytic or stoichiometric manner, respectively, is strongly demanded from an economical point of view in large-scale synthesis. Thus, the present method meeting these requirements is highly superior to existing approaches.[4,5a]

Experimental Section

General Procedures and Materials. IR spectra were measured in KBr on a JASCO FT/IR-5300 spectrometer. UV spectra were taken in MeOH on a JASCO V-550 spectrometer. NMR spectra were obtained in $CDCl_3$ on a JEOL α-400 instrument. The ^{1}H and ^{31}P chemical shifts are described as δ values in parts per million relative to $(CH_3)_4Si$ and 85% H_3PO_4, respectively. High-performance liquid chromatography (HPLC) was achieved using a COSMOSIL 5C18-MS

(nacalai tesque, ODS-5μm) column on a JASCO 88-PU chromatograph with a JASCO 870-UV detector. Elemental analysis was performed at the Graduate School of Human Informatics, Nagoya University. The nucleosides, **1**,[11c] **2**,[12] **3**,[17] and **22**,[11d] and the nucleoside phosphoramidites, **4**,[8a] **5**,[8a] **6**,[8a] **7**,[8a] **9**,[5b] **10**,[5b] **14**,[8a] **23**,[13] and **24**,[11d] were prepared by the reported methods. The amidites were stored in a refrigerator. Acetonitrile used as the reaction solvent was distilled from CaH_2. Column chromatography was achieved with E. Merck Kieselgel 60 (70−230 mesh) deactivated by adding 6% water. Solvents for the chromatography were used after simple distillation of the commercially supplied ones. 5-(*p*-Nitrophenyl)-1*H*-tetrazole (Lancaster) and molecular sieves 13X powder (Aldrich) were used as commercially supplied. The products, **18**,[12] **25**,[12] and **26**,[11d] are known the in the literature. The spectral and physical data of the new compounds, **8**, **11**−**17**, **19**, and **21**, are as follows.

5′-*O*-(*p*,*p*′-Dimethoxytrityl)thymidine 3′-(Allyl *N*,*N*-dimethylphosphoramidite) (8). To a suspension of 5′-*O*-(*p*,*p*′-dimethoxytrityl)-thymidine (5.00 g, 9.19 mmol), (allyloxy)bis(dimethylamino)phosphine (1.49 g, 9.19 mmol), and molecular sieves 3A (200 mg) in acetonitrile (50 mL) was added *N*-methylimidazolium triflate[18] (1.07 g, 4.60 mmol) in five portions. The resulting mixture was stirred at 25 °C for 1.5 h and then poured into ether (200 mL). The organic solution was washed with brine (100 mL × 2) and concentrated to give **8** (6.09 g, 98% yield) as a colorless solid: IR 1690, 1609, 1510, 1464 cm^{-1}; UV λ_{max} 235 (ϵ 28 300), 268 nm (14 100); ^{1}H NMR δ 1.44 (s, 3H), 2.24−2.35 (m, 1H), 2.41−2.70 (m, 7H), 3.28−3.35 (m, 1H), 3.46−3.51 (m, 1H), 3.79 (s, 6H), 4.03−4.07 (m, 1H), 4.11−4.19 (m, 2H), 4.66−4.71 (m, 1H), 5.08−5.28 (m, 2H), 5.77−5.95 (m, 1H), 6.38−6.44 (m, 1H), 6.83 (d, 4H, J = 9.3 Hz), 7.21−7.41 (m, 10H), 7.60, 7.61 (2 s, 1H); ^{31}P NMR δ −148, −147. This compound is too labile to be subjected to the mass spectral and elemental analyses. The crude product was used for further reaction immediately after preparation without purification.

Preparation of Allyl [5′-*O*-(*p*,*p*′-Dimethoxytrityl)thymidylyl]-(3′−5′)-3′-*O*-[(allyloxy)carbonyl]thymidine (18). The Typical Procedure for the Synthesis of Dinucleoside Phosphates. To a solution of the nucleoside **2** (8.16 g, 25.0 mmol), the amidite **7** (17.9 g, 26.3 mmol), and NPT (243 mg, 1.25 mmol) in acetonitrile (50 mL) was added molecular sieves (MS) 13X (20.0 g). The resulting heterogeneous mixture was vigorously stirred at 40 °C for 60 min. To the reaction mixture were added a 1.0 M solution of bis(trimethylsilyl) peroxide (TMSOOTMS) in dichloromethane (30.0 mL, 30.0 mmol) and a 0.25 M solution of trimethylsilyl triflate (TMSOTf) in dichloromethane (6.00 mL, 1.50 mmol),[10] and stirring was continued for 10 min. After removal of the molecular sieves by filtration, the filtrate was concentrated to give an oil, which was dissolved in a 9:1 mixture of ethyl acetate and hexane (60 mL). The solution was passed through a silica gel pad (20 g) and washed with a 9:1 mixture of ethyl acetate and hexane (40 mL). Concentration of the combined filtrate and washing afforded **18** (24.1 g, 99% yield) as a colorless amorphous solid.

The dinucleoside phosphates, **11**−**17**, were prepared in a similar manner.

Allyl [*N*^{6}-[(Allyloxy)carbonyl]-5′-*O*-(*p*,*p*′-dimethoxytrityl)-2′-deoxyadenylyl]-(3′−5′)-*N*^{4},3′-*O*-bis[(allyloxy)carbonyl]-2′-deoxycytidine (11): a colorless amorphous solid; IR 1749, 1671, 1613, 1584, 1508, 1464 cm^{-1}; UV λ_{max} 238 (ϵ 26 900), 268 nm (18 300); ^{1}H NMR δ 2.09−2.19 (m, 1H), 2.72−2.30 (m, 2H), 3.12−3.19 (m, 1H), 3.34−3.45 (m, 2H), 3.72, 3.73 (2 s, 6H), 4.32−4.61 (m, 10H), 4.71 (d, 2H, J = 4.6 Hz), 5.15−5.36 (m, 10H), 5.82−5.96 (m, 4H), 6.21−6.26 (m, 1H), 6.43−6.47 (m, 1H), 6.74 (d, 4H, J = 8.8 Hz), 7.14−7.26 (m, 10H), 7.99−8.02 (m, 1H), 8.17 (d, 1H, J = 10.7 Hz), 8.60−8.62 (m, 2H), 8.84−9.89 (br s, 1H); ^{31}P NMR δ −1.1, −1.0. Anal. Calcd for $C_{55}H_{59}N_8O_{17}P$: C, 58.20; H, 5.24; N, 9.87. Found: C, 58.21; H, 5.27; N, 9.97.

Allyl [*N*^{4}-[(Allyloxy)carbonyl]-5′-*O*-(*p*,*p*′-dimethoxytrityl)-2′-deoxycytidylyl]-(3′−5′)-*N*^{4},3′-*O*-bis[(allyloxy)carbonyl]-2′-deoxycytidine (12): a colorless amorphous solid; IR 1751, 1653, 1561, 1503, 1402 cm^{-1}; UV λ_{max} 238 (ϵ 20 700), 293 nm (6700); ^{1}H NMR δ 2.15−2.25 (m, 1H), 2.30−2.36 (m, 1H), 2.76−2.91 (m, 2H), 3.37−3.48 (m, 2H), 3.79, 3.80 (2 s, 6H), 4.28−4.67 (m, 12H), 5.07−5.39 (m, 10H), 5.82−5.97 (m, 4H), 6.19−6.27 (m, 2H), 6.83−6.85 (m, 4H), 7.03−7.06 (m, 1H), 7.24−7.36 (m, 11H), 7.96−8.22 (m, 2H), 8.44−8.66 (br s, 1H); ^{31}P NMR δ −1.3, −1.1. Anal. Calcd for $C_{54}H_{59}N_6O_{18}P$: C, 58.38; H, 5.35; N, 7.56 . Found: C, 58.25; H, 5.26; N, 5.77.

Allyl [*N*^{2}-[(Allyloxy)carbonyl]-*O*^{6}-allyl-5′-*O*-(*p*,*p*′-dimethoxytrityl)-2′-deoxyguanylyl]-(3′−5′)-*N*^{4},3′-*O*-bis[(allyloxy)carbonyl]-2′-deoxycytidine (13): a colorless amorphous solid; IR 1752, 1698, 1609, 1510, 1460, 1418 cm^{-1}; UV λ_{max} 237 (ϵ 59 300), 269 nm (50 200); ^{1}H NMR δ 2.10−2.22 (m, 1H), 2.69−2.89 (m, 2H), 3.08−3.20 (m, 1H), 3.35−3.51 (m, 2H), 3.75, 3.76 (2 s, 6H), 4.33−4.39 (m, 4H), 4.50−4.68 (m, 8H), 5.07 (d, 2H, J = 5.3 Hz), 5.17−5.49 (m, 12H), 5.82−6.01 (m, 4H), 6.10−6.20 (m, 1H), 6.28 (t, 1H, J = 6.8 Hz), 6.33−6.39 (m, 1H), 6.72−6.77 (m, 4H), 7.16−7.37 (m, 10H), 7.53, 7.66 (2 s, 1H), 7.76−7.85 (br s, 1H), 7.93, 7.97 (2 s, 1H), 7.93, 7.97 (2 d, 1H, J = 11.7 Hz); ^{31}P NMR δ −1.1, −1.0. Anal. Calcd for $C_{58}H_{63}N_8O_{18}P$: C, 58.48; H, 5.33; N, 9.41. Found: C, 58.25; H, 5.33; N, 9.51.

Allyl [5′-*O*-(*p*,*p*′-Dimethoxytrityl)thymidylyl]-(3′−5′)-*N*^{4},3′-*O*-bis[(allyloxy)carbonyl]-2′-deoxycytidine (14): a colorless amorphous solid; IR 1750, 1686, 1561, 1508, 1460 cm^{-1}; UV λ_{max} 237 (ϵ 33 700), 272 nm (14 400); ^{1}H NMR δ 1.41 (s, 3H), 2.07−2.17 (m, 1H), 2.39−2.47 (m, 1H), 2.60−2.65 (m, 1H), 2.83 (dt, 1H, J = 4.9, 15.1 Hz), 3.38 (dd, 1H, J = 11.2, 11.7 Hz), 3.50−3.55 (m, 1H), 3.79, 3.90 (2 s, 6H), 4.22−4.38 (m, 3H), 4.44−4.66 (m, 7H), 5.12−5.39 (m, 8H), 5.79−5.97 (m, 3H), 6.26 (t, 1H, J = 6.5 Hz), 6.41, 6.42 (2 s, 1H, J = 8.8 Hz), 6.84 (d, 4H, J = 8.3 Hz), 7.20−7.39 (m, 10H), 7.54, 7.57 (2 s, 1H), 7.98, 8.04 (2 d, 1H, J = 7.5 Hz), 8.09 (s, 1H), 9.11, 9.16 (2 s, 1H); ^{31}P NMR δ −1.1, −1.0. Anal. Calcd for $C_{51}H_{56}N_5O_{17}P$: C, 58.79; H, 5.42; N, 6.72. Found: C, 58.78; H, 5.44; N, 6.70.

Allyl [*N*^{6}-[(Allyloxy)carbonyl]-5′-*O*-(*p*,*p*′-dimethoxytrityl)-2′-deoxyadenylyl]-(3′−5′)-3′-*O*-[(allyloxy)carbonyl]thymidine (15): a colorless amorphous solid; IR 1752, 1698, 1609, 1510, 1460, 1418 cm^{-1}; UV λ_{max} 237 (ϵ 39 200), 269 nm (33 700); ^{1}H NMR δ 1.91, 1.94 (2 s, 3H), 2.20−2.31 (m, 1H), 2.47−2.53 (m, 1H), 2.49, 2.50 (dt, 1H, J = 5.8, 7.3 Hz), 2.76−2.84 (m, 1H), 3.09−3.18 (m, 1H), 3.37−3.50 (m, 1H), 3.76, 3.77 (2 s, 6H), 4.21−4.41 (m, 4H), 4.51−4.69 (m, 4H), 4.76 (d, 2H, J = 4.4 Hz), 5.18−5.45 (m, 8H), 5.85−6.05 (m, 3H), 6.31−6.38 (m, 1H), 6.45−6.52 (m, 1H), 6.77−6.80 (m, 4H), 7.19−7.41 (m, 10H), 8.65, 8.66 (2 s, 1H), 8.82, 8.83 (2 s, 1H), 8.87−9.91 (br s, 1H), 9.92−10.01 (br s, 1H); ^{31}P NMR δ −1.5, −1.1. Anal. Calcd for $C_{52}H_{56}N_7O_{16}P$: C, 58.59; H, 5.29; N, 9.20. Found: C, 58.59; H, 5.26; N, 9.25.

Allyl [*N*^{4}-[(Allyloxy)carbonyl]-5′-*O*-(*p*,*p*′-dimethoxytrityl)-2′-deoxycytidylyl]-(3′−5′)-3′-*O*-[(allyloxy)carbonyl]thymidine (16): a colorless amorphous solid; IR 1748, 1688, 1508, 1466 cm^{-1}; UV λ_{max} 235 (ϵ 23 600), 267 nm (11 100); ^{1}H NMR 1.87, 1.89 (2 s, 3H), 2.18−2.52 (m, 3H), 2.82−2.94 (m, 1H), 3.33−3.49 (m, 2H), 3.77, 3.78 (2 s, 6H), 4.15−4.69 (m, 10H), 5.07−5.42 (m, 8H), 5.84−6.01 (m, 3H), 6.17−6.38 (m, 2H), 6.82−6.84 (m, 4H), 6.95 (d, 1H, J = 5.7 Hz), 7.17−7.41 (m, 10H), 8.02−8.08 (m, 1H), 8.23−8.47 (br s, 1H), 9.55−9.78 (br s, 1H); ^{31}P NMR δ −1.4, −1.1. Anal. Calcd for $C_{51}H_{56}N_5O_{17}P$: C, 58.79; H, 5.42; N, 6.72. Found: C, 58.56; H, 5.45; N, 6.88.

Allyl [*N*^{2}-[(Allyloxy)carbonyl]-*O*^{6}-allyl-5′-*O*-(*p*,*p*′-dimethoxytrityl)-2′-deoxyguanylyl]-(3′−5′)-3′-*O*-[(allyloxy)carbonyl]thymidine (17): a colorless amorphous solid; IR 1752, 1698, 1609, 1510, 1460, 1418 cm^{-1}; UV λ_{max} 237 (ϵ 26 700), 269 nm (25 400); ^{1}H NMR δ 1.92 (s, 3H), 2.21−2.38 (m, 1H), 2.42−2.56 (m, 1H), 2.69−2.81 (m, 1H), 3.02−3.12 (m, 1H), 3.32−3.38 (m, 1H), 3.44−3.48 (m, 1H), 3.75, 3.76 (2 s, 6H), 4.20−4.41 (m, 4H), 4.50−4.68 (m, 6H), 5.07 (d, 2H, J = 4.9 Hz), 5.22−5.49 (m, 10H), 5.81−6.02 (m, 3H), 6.09−6.18 (m, 1H), 6.28−6.32 (m, 1H), 6.39 (t, 1H, J = 6.3 Hz), 6.72−6.79 (m, 4H), 7.16−7.39 (m, 10H), 7.60, 7.73 (2 s, 1H), 7.91, 7.92 (2 s, 1H), 8.86−9.02 (2 br s, 1H); ^{31}P NMR δ −1.4, −1.1. Anal. Calcd for $C_{55}H_{60}N_7O_{17}P$: C, 58.87; H, 5.39; N, 8.74. Found: C, 58.67; H, 5.27; N, 8.94.

Preparation of Allyl [*N*^{2}-[(Allyloxy)carbonyl]-*O*^{6}-allyl-2′-deoxyguanylyl]-(3′−5′)-3′-*O*-[(allyloxy)carbonyl]thymidine (19). A heterogeneous mixture of **2** (978 mg, 3.00 mmol), **6** (2.52 g, 3.15 mmol), NPT (28.7 mg, 150 μmol), and MS 13X (2.50 g) in acetonitrile (10 mL) was vigorously stirred at 40 °C for 60 min. To this mixture were added a 1.0 M solution of TMSOOTMS in dichloromethane (4.00 mL, 4.00 mmol) and a 0.25 M solution of trimethylsilyl triflate in dichloromethane (0.80 mL, 200 μmol), and stirring was continued for

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10 min. MS 13X was removed by filtration, and the filtrate was concentrated to give a gummy oil. This material was dissolved in 3% dichloroacetic acid in a 3:97 mixture of methanol and dichloromethane (100 mL)[19] and stirred for 10 min. The solution was poured into an aqueous sodium hydrogen carbonate solution (200 mL). The separated organic layer was washed with brine (100 mL) and concentrated to the half-volume. The resulting solution was poured into a 1:1 mixture of diethyl ether and hexane (500 mL) to precipitate **19** (2.34 g, 95%) as a colorless powder, which was collected by filtration: IR 1751, 1701, 1609, 1534, 1462, 1414 cm^{-1}; UV λ_{max} 215 (ϵ 30 500), 267 nm (19 200); ^{1}H NMR δ 1.94, 1.95 (2 s, 3H), 2.30−2.44 (m, 1H), 2.47−2.62 (m, 2H), 3.17−3.29 (m, 1H), 3.78−3.99 (m, 3H), 4.27−4.31 (m, 1H), 4.32−4.44 (m, 3H), 4.60−4.66 (m, 4H), 4.69 (d, 2H, J = 5.4 Hz), 4.83 (ddd, 1H, J = 26.6, 10.0, 3.4 Hz), 5.09 (d, 2H, J = 5.8 Hz), 5.23−5.49 (m, 10H), 5.87−6.02 (m, 2H), 6.14 (ddt, 1H, J = 10.5, 17.4, 5.4 Hz), 6.24−6.33 (m, 2H), 7.37, 7.39 (2 s, 1H), 7.61 (s, 1H), 7.89, 7.90 (2 s, 1H), 8.64−8.79 (2 br s, 1H); ^{31}P NMR δ −1.7, −1.0. Anal. Calcd for $C_{34}H_{42}N_7O_{15}P$: C, 49.82; H, 5.16, N, 11.96. Found: C, 49.95; H, 5.08; N, 11.95.

Synthesis of d(5′CTACCTGT3′) (21). Elongation of the 5′-O-free nucleotide **19** was achieved via a procedure similar to that for the formation **19** described above to give the protected octamer **20** as a colorless powder (5.45 g, 57% overall yield). The whole crude product was dissolved in THF (50 mL) containing degassed H_2O (10 mL), and to this were added $Pd[P(C_6H_5)_3]_4$ (593 mg, 513 μmol), $P(C_6H_5)_3$ (83.1 mg, 313 μmol), and diethylammonium hydrogen carbonate (5.54 g, 41.0 mmol). The solution was stirred at 25 °C for 3 h and then poured into dichloromethane (50 mL) with vigorous stirring to give diethylammonium salts of **21** (4.90 g, 115 800 OD_{260}, 52% overall yield from **19**) as a colorless solid: ^{31}P NMR δ −0.77. The HPLC analysis of the product with a 0.1 M triethylammonium acetate buffer containing 5−15% acetonitrile (v/v) (linear gradient in 30 min) indicated that the purity of **21** is >98%.

Enzymatic Digestion of the Octamer 21 and HPLC Analysis of the Resulting Products. A mixture of the purified octamer **21** (0.40 OD_{260}) in H_2O (10 μL), snake venom phosphodiesterase (1 μL, 0.1 unit), bacterial alkaline phosphatase (10 μL, 2.5 units), 300 mM Tris−HCl (5 μL), and 300 mM $MgCl_2$ (2.5 μL) was incubated at 37 °C for 24 h and then heated at 90 °C for 1 h. The aliquot (5 μL) was subjected directly to HPLC analysis with a 9:1 mixture of H_2O−MeOH as eluent (1 mL/min, 40 °C), which showed four peaks due to dC (t_R = 3.5 min), dG (4.6 min), T (5.8 min), and dA (7.0 min) in a 3.0:0.9:3.1:0.9 ratio. No *N*-AOC-protected nucleosides were detected.

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Supporting Information Available: Characterization data including ^{1}H and ^{31}P NMR spectral charts for all new compounds (20 pages). See any current masthead page for ordering or Internet access instructions.

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