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Introduction of 3′-Terminal Nucleosides Having a Silyl-Type Linker into Polymer Supports without Base Protection

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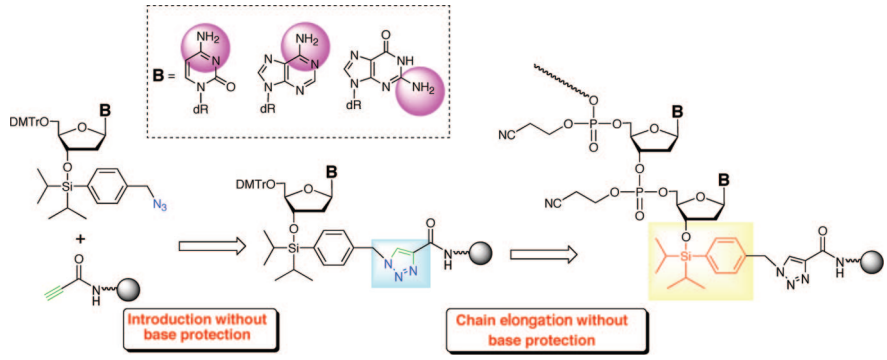

New 3′-terminal deoxyribonucleoside-loading reagents having a silyl-type linker were developed. They were effectively introduced into polymer supports under the conditions of Huisgen [3 + 2] cycloaddition without base protection. Moreover, four unmodified DNA oligomers d[TACCTAAATCCAX] (X = T, A, C, and A) and a base-labile modified DNA 12mer d[A*C*T*C*C*GT*C*T*A*C*G] **16** (A* = 6-*N*-acetyl-8-aza-7-deaza-2′-deoxyadenosine, C* = 4-*N*-acetyl-2′-deoxyctydine, T* = 2-thio-T) were successfully synthesized by cleavage of the silyl-type linker using Bu_4NF under neutral conditions in our N-unprotected phosphoramidite method. In this paper, we also report a new reaction of chlorination of cytosine base using 1,3-dichloro-5,5-dimethylhydantoin.

Introduction

The fields of chemical biology, biotechnology, and molecular biology have progressed rapidly following the development of the phosphoramidite method as a practical procedure for DNA and RNA synthesis by Caruthers and co-workers,[1] largely because researchers can easily obtain oligonucleotides having the desired sequences. Not only unmodified oligonucleotides but also oligonucleotides having various functional groups[2] are required for current research because the diversification of studies in these fields has progressed considerably. However, some useful oligonucleotides having base-labile functional groups, such as aminoacylated RNAs,[3] N-acylated oligonucleotides,[4] and 2′-*O*-acyloxymethyl RNAs,[5] cannot be synthesized via solid-phase synthesis using the standard phosphoramidite procedure.[1] This is because the latter method uses ammonium

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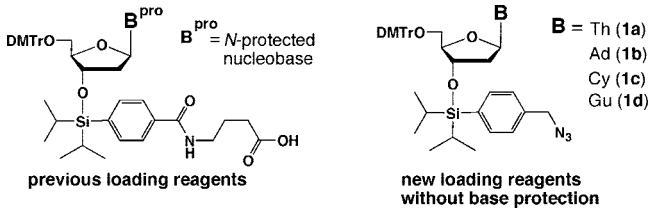
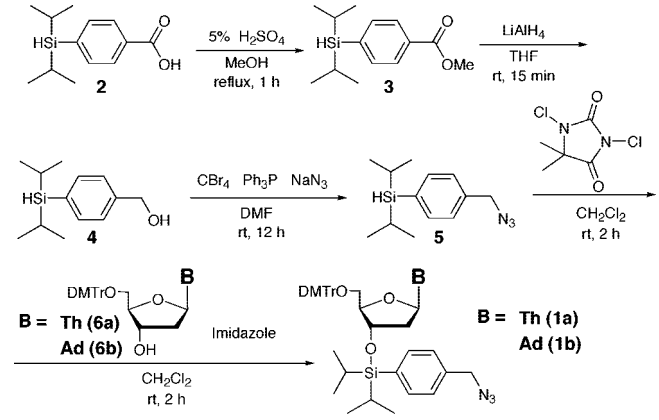


FIGURE 1. Skeletons of previous N-protected loading reagents and loading reagents **1a**−**d** containing a silyl linker without base protection.

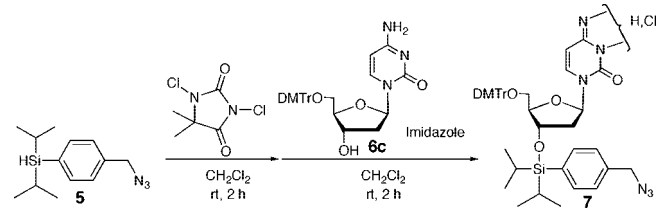
hydroxide for the removal of the protecting groups and their release from polymer supports; base-labile functional groups are decomposed by this treatment. To overcome this serious problem, we developed a so-called "activated phosphite method"[6] for O-selective condensation using 3′-terminal deoxyribonucleoside-loading reagents having a silyl-type linker[6b,7] that can be cleaved under neutral conditions. Using this approach, we succeeded in synthesizing oligonucleotides containing base-labile deoxynucleoside derivatives such as N^4-acetyl-2′-deoxycytidine or N^6-acetyl-8-aza-7-deaza-2′-deoxyadenosine in good yields. Nevertheless, there is still a problem: the amino groups of the previous loading reagents[7] must be protected to avoid side reactions on the nucleobases since the loading reaction requires a coupling between the amino group on the polymer supports and the carboxyl group of the silyl linker attached to the 3′-OH group of the nucleosides (Figure 1). Therefore, in the synthesis of oligonucleotides incorporating base-labile functional groups using our N-unprotected phosphoramidite approach, we needed to treat the resin with methylamine to remove the base-protecting group from the 3′-terminal N-protected deoxynucleoside-loaded resin. Unfortunately, we observed that 6−18% of the once-loaded deoxynucleoside was partially eliminated from the resins by treatment with methylamine. In addition, various 3′-terminal nucleosides having base-labile functional groups at the same position cannot be used because they decompose under the treatment with methylamine, such as 2′-O-modified nucleosides or 5-modified pyrimidines. If a 3′-terminal nucleoside component can be introduced into the resin without base protection, the problems just described can be resolved because then the first deprotection step would be unnecessary.

Copper-catalyzed Huisgen [3 + 2] cycloadditions between azido and alkyne groups, which were developed by Meldal[8] and Sharpless,[9] have been widely used. Recently, some groups have reported that the reaction could be used to functionalize alkyne-modified oligonucleotides without base protection in good yield.[10] Those results indicated that the N-unprotected loading reagents **1a**−**d** having an azido group (Figure 1) could be efficiently incorporated into alkyne-modified resins without side reactions. We report the synthesis of the reagents **1a**−**d** and their introduction into highly cross-linked polystyrene[11] (HCP) resins without base protection.

SCHEME 1. Synthesis of T- and dA-Loading Reagents 1a and 1b without Base Protection



SCHEME 2. Silylation of DMT-C 6c Using the Silane Derivative 5 in the Presence of 1,3-Dichloro-5,5-dimethylhydantoin



Results and Discussion

Scheme 1 shows an efficient synthetic route to the T- and A-loading reagents **1a** and **1b**, respectively. Compound **2** was first esterified to increase the solubility in THF. Subsequently, the resulting ester **3** was reduced to alcohol **4** using $LiAlH_4$ in THF. The introduction of an azido group was carried out using $CBr_4-Ph_3P-NaN_3$[12] to obtain compound **5** in 78% yield. This product was converted to a silyl chloride derivative by treatment with 2 equiv of 1,3-dichloro-5,5-dimethylhydantoin for 2 h. The in situ generated silyl chloride derivative was allowed to react with 5′-*O*-DMTr-T **6a** and 5′-*O*-DMTr-dA **6b** without base protection in the presence of imidazole to produce compounds **1a** and **1b** in 93 and 65% yields, respectively. However, when 5′-*O*-DMTr-dC was used under the same conditions, instead of the desired 3′-O-silylated product, an unexpected species was obtained (Scheme 2). Interestingly, the NMR and mass data of this unexpected product suggested that not only silylation of the 3′-hydroxyl group but also chlorination of the cytosine base had occurred. Since the 5- and 6-protons of the cytosine base were clearly observed in the ^1H NMR spectrum (see Supporting Information), there was a possibility that the chlorination had occurred on the exo 4-amino group or heterocyclic 3-nitrogen. Such an unusual chlorination of the cytosine base has not been reported until now. To determine the location where the chloro group was introduced, a similar reaction was carried out using a ^{15}N-labeled 3′,5′-*O*-bis(TBDMS)-dC derivative **8** (Scheme 3). We found that the amino group of **8** readily reacted with 1,3-dichloro-5,5-dimethylhydantoin within 10 min to produce a similar base-chlorinated product **9** that could be isolated as a stable material in 93% yield by silica gel column chromatography. Since the peak of an imino proton observed at around

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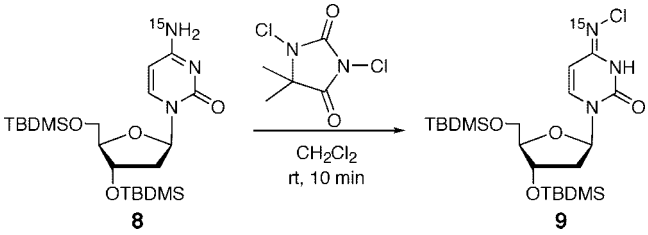
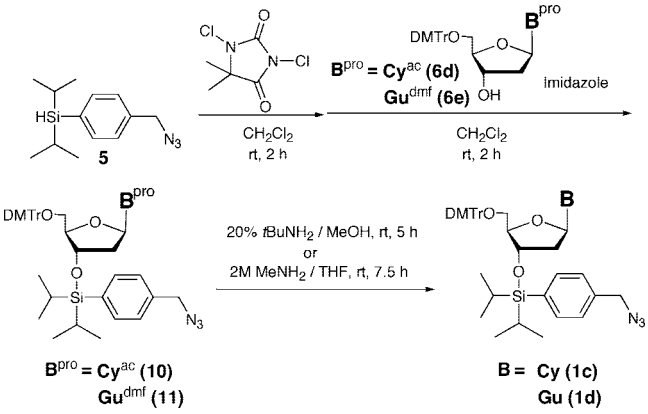
SCHEME 3. Chlorination of ^{15}N-Labeled dC Derivative 8 Using 1,3-Dichloro-5,5-dimethylhydantoin



SCHEME 4. Synthesis of dC- and dG-Loading Reagents 1c and 1d Using Acetyl or DMF Groups

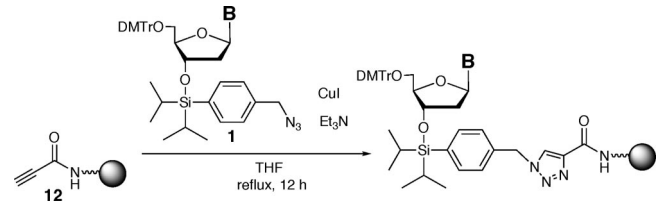


8.5 ppm was a singlet rather than a doublet, we concluded that the chlorinated compound has a 15N−Cl bond instead of a 15N−1H bond at the 4-position. Although this chlorination was an undesired side reaction in the present study, the facile selective N-chlorination of the cytosine moiety might be very interesting and useful for obtaining synthetic intermediates in nucleoside and oligonucleotide chemistry.

We had previously carried out efficient silylation of an *N*-acetyl dC derivative in the presence of 1,3-dichloro-5,5-dimethylhydantoin. This result suggested that the protection for the amino group of the cytosine base using an acetyl group could completely avoid the undesired chlorination described previously. Therefore, 5′-*O*-DMTr-4-*N*-acetyl-dC **6d** was employed for introducing the silyl residue into its 3′-position (Scheme 4). This reaction yielded the desired product, which in turn was treated with 20% *t*BuNH$_2$/MeOH. As a result, the dC-loading reagent **1c** was obtained in 81% yield via two steps. On the other hand, for the synthesis of the dG-loading reagent **1d**, the guanine base was protected with an *N*,*N*-dimethylaminomethylene (DMF) group to simplify purification of the target compound. Thus, the 3′-O-silylated product was easily obtained, and the DMF group was selectively removed by treatment with 2 M methylamine/THF[13] to produce compound **1d** in 50% yield via two steps.

Next, we examined the efficiency of the introduction of the 3′-terminal nucleoside residues into polymer supports using the loading reagents **1a**−**d** under various conditions (Scheme 5). We found that the loading reagents could be efficiently introduced into the alkyne-loaded HCP resin **12** (the loading amount of the terminal amino group: 34 μmol/g) in the presence of CuI and Et$_3$N under reflux for 12 h. The T-, dA-, dC-, and dG-loading amounts were estimated by DMTr cation assay as

SCHEME 5. Introduction of 3′-Terminal Nucleoside-Loading Reagents 1 into HCP Resins by Copper-Catalyzed Huisgen [3 + 2] Cycloaddition



27, 30, 27, and 34 μmol/g, respectively. For the first time, we could efficiently introduce an N-unprotected nucleoside into polymer supports. Moreover, we also found by the DMTr cation assay that these deoxynucleosides could be completely released from the resins by treatment with a 1 M THF solution of TBAF−AcOH for 6 h under neutral conditions, although release of the thymidine residue from the resin **13a** by treatment with a 0.2 M THF solution of Et$_3$N−3HF was very slow (t_{comp} = 24 h), as shown in Figure 2.

Further, we demonstrated the synthesis of four DNA oligomers d[TACCTAAATCCAX] (X = T, A, C, and A) by the activated phosphite method (Scheme 6). Each chain elongation was carried out using 6-nitro-1-hydroxybenzotriazole (HO^{n}Bt) in the presence of benzimidazolium triflate[14] (BIT) as an activator on the resins **13a**−**d** containing a silyl linker. After chain elongation, selective removal of the 2-cyanoethyl groups of the internucleotidic phosphates was carried out by treatment with 1 M DBU in CH$_3$CN for 1 min, and the successive removal of the 5′-terminal DMTr group was carried out by treatment with 3% trichloroacetic acid in CH$_2$Cl$_2$. Finally, the desired DNA oligomers **15a**−**d** were released from the resin by treatment with 1 M Bu$_4$NF (TBAF)−AcOH in THF at room temperature. Each complete release of DNA oligomers required a prolonged time (24 h; Figure 3), compared with that of only deoxynucleosides. This decrease of reactivity in the Si−O bond cleavage might result from the effect of anionic charges of the phosphate groups. The crude products were analyzed by anion-exchange HPLC (Figure 4). The purification produced the oligomers **15a**−**d** in 44, 37, 52, and 33% yields, respectively. These oligomers were characterized by MALDI-TOF mass spectrometry. These results show that the 3′-terminal 2′-deoxynucleoside-loaded HCP resins prepared using the loading reagents **1a**−**d** are very useful for the synthesis of DNA oligomers by the activated phosphite method.

Finally, we carried out the synthesis of a base-labile modified DNA 12mer d[A*C*T*C*C*GT*C*T*A*C*G] **16** (A* = 6-*N*-acetyl-8-aza-7-deaza-2′-deoxyadenosine: ac^{6}az^{8}c^{7}A, C* = 4-*N*-acetyl-2′-deoxyctydine: ac^{4}C, T* = 2-thio-T: s^{2}T).[4c] In our recent study, it turned out that introduction of the modified base, ac^{6}az^{8}c^{7}A, ac^{4}C, and s^{2}T into DNA oligomers increased the hybridization affinity for the cDNA oligomers and their base recognition ability compared to the unmodified adenine base. However, significant elimination of the acetyl group occurred upon treatment with ammonium hydroxide for deprotection and release of the DNA oligomer in the standard procedure for DNA synthesis. Therefore, we carried out the synthesis of the modified

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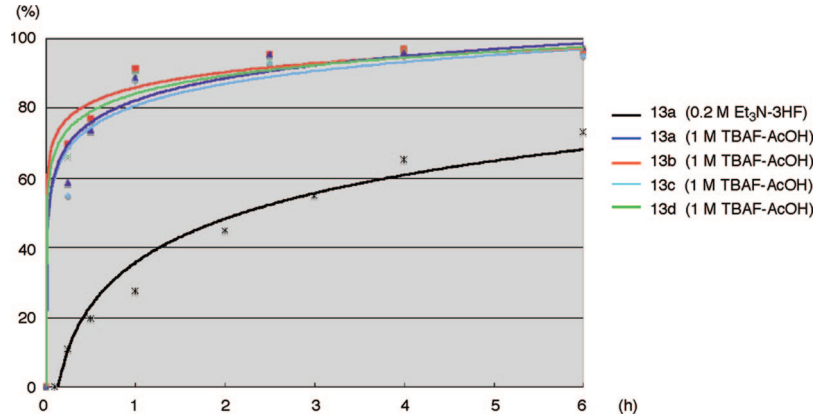


FIGURE 2. Release of nucleosides from the HCP resins **13a−d** by treatment with 0.2 M THF solution of Et_3N-3HF or 1 M THF solution of TBAF−AcOH.

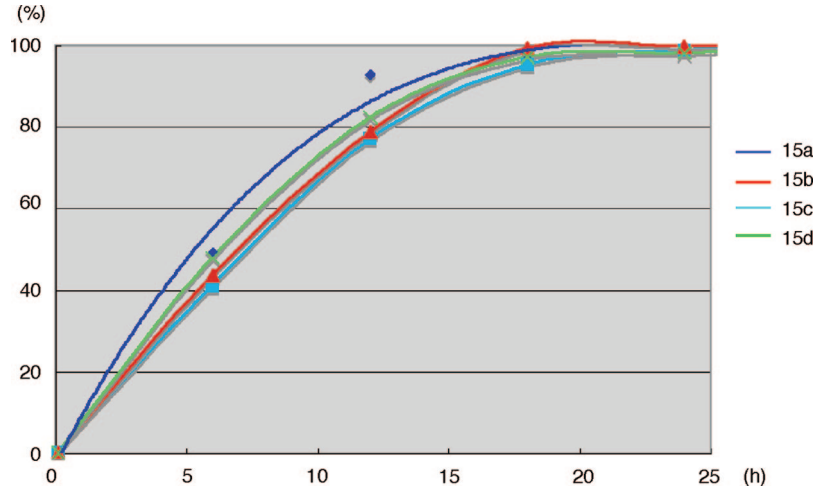
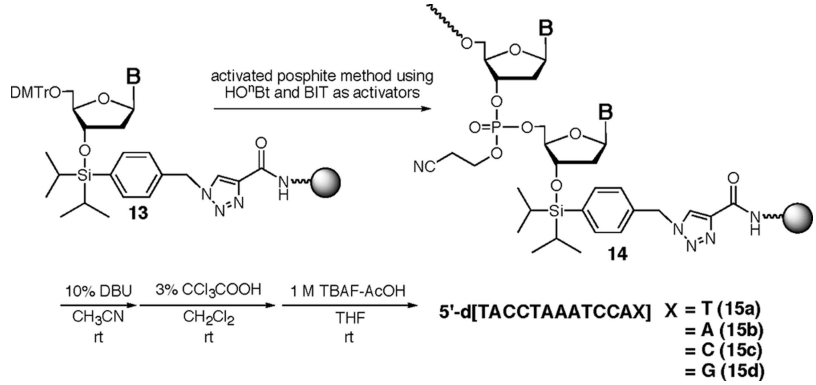


FIGURE 3. Release of oligonucleotides **15a−d** from the HCP resins by treatment with 1 M THF solution of TBAF−AcOH.

SCHEME 6. Synthesis of DNA Oligomers in the Activated Phosphite Method with 3′-Terminal Nucleoside-Loaded HCP Resins 13 Containing a Silyl Linker



DNA **16** by the activated phosphite method using a G-loaded HCP resin **13d** with a silyl linker. As a result, the modified DNA **16** could be synthesized without decomposition and the oligomer **16** was obtained in 41% yield (Figure 4e). This oligomer was characterized by MALDI-TOF mass spectrometry.

Conclusion

We report the first synthesis of 3′-terminal N-unprotected deoxynucleoside-loading reagents **1a−d** having a silyl linker. We also found that these reagents can be effectively introduced into HCP resins without side reactions under the conditions of Huisgen [3 + 2] cycloaddition. Moreover, we succeeded in synthesizing DNA oligomers using the nucleoside-loaded resins having a silyl linker by the activated phosphite method, without using a standard procedure such as the addition of ammonium hydroxide for release of oligomers from resins. These results have encouraged us to study the synthesis of base-labile oligonucleotides having functional groups at the 3′-terminal position, such as aminoacylated RNAs. Further studies are now underway in this direction.

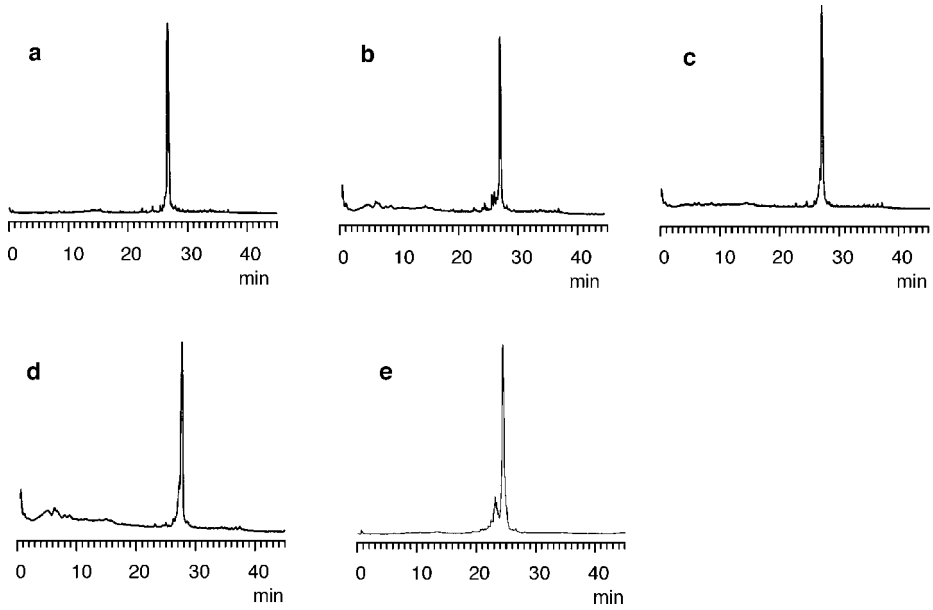


FIGURE 4. Anion-exchange HPLC profiles of the crude mixtures obtained by the activated phosphite method: (a) d[TACCTAAATCCAT] **15a**, (b) d[TACCTAAATCCAA] **15b**, (c) d[TACCTAAATCCAC] **15c**, (d) d[TACCTAAATCCAG] **15d**, and (e) d[A*C*T*C*C*GT*C*T*A*C*G] **16**, A* = 6-*N*-acetyl-8-aza-7-deaza-A, C* = 4-*N*-acetyl-C, T* = 2-thio-T.

Experimental Section

Synthesis of Compound 3. Compound **2** (9 g, 38 mmol) was added to 5% H_2SO_4 in MeOH (300 mL). After being refluxed with stirring for 1 h, the mixture was partitioned between CH_2Cl_2 (300 mL) and brine (300 mL). The organic phase was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (150 g) with hexane/AcOEt (100:0−95:5, v/v) to give **3** (7.2 g, 76%): ^{1}H NMR ($CDCl_3$) δ 0.93−1.06 (m, 12H), 1.18−1.27 (m, 2H), 3.90 (s, 3H), 3.96 (t, 1H, J = 3.2 Hz), 7.58 (d, 2H, J = 8.1 Hz), 7.98 (d, 2H, J = 8.1 Hz); ^{13}C NMR ($CDCl_3$) δ 10.6, 18.5, 18.6, 52.2, 128.1, 128.2, 128.3, 130.5, 140.6, 167.1. Anal. Calcd for $C_{14}H_{22}O_2Si$: C, 67.15; H, 8.86. Found: C, 67.27; H, 8.68.

Synthesis of Compound 4. $LiAlH_4$ (1.76 g, 46.5 mmol) was dissolved in dry THF (50 mL). To the solution was added dropwise the dry THF solution (50 mL) of compound **3** (10.6 g, 42.3 mmol) at 0 °C. After the mixture was stirred at room temperature for 15 min, AcOEt (20 mL) was added dropwise to the mixture. The mixture was filtered using a Celite bed. The mixture was partitioned between CH_2Cl_2 (500 mL) and 0.2 M aqueous HCl (300 mL). The organic phase was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (150 g) with hexane/AcOEt (100:0−90:10, v/v) to give **4** (9.4 g, 85%): ^{1}H NMR ($CDCl_3$) δ 0.96−1.07 (m, 12H), 1.17−1.23 (m, 2H), 3.09 (br s, 1H), 3.94 (t, 1H, J = 3.2 Hz), 4.58 (s, 2H), 7.29 (d, 2H, J = 7.6 Hz), 7.48 (d, 2H, J = 7.6 Hz); ^{13}C NMR ($CDCl_3$) δ 10.7, 18.4, 18.6, 64.8.2, 126.0, 132.9, 135.4, 141.6. Anal. Calcd for $C_{13}H_{22}OSi$: C, 70.21; H, 9.97. Found: C, 69.93; H, 10.00.

Synthesis of Compound 5. Compound **4** (440 mg, 2 mmol) was rendered anhydrous by repeated coevaporation with dry CH_3CN and finally dissolved in dry DMF (4 mL). To the solution were added CBr_4 (680 m g, 2.1 mmol), PPh_3 (540 mg, 2.1 mmol), and NaN_3 (650 mg, 10 mmol). After the mixture was stirred at room temperature for 12 h, the mixture was partitioned between AcOEt (50 mL) and brine (50 mL). The organic phase was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (10 g) with hexane/AcOEt (100:0−90:10, v/v) to give **4** (380 mg, 78%): ^{1}H NMR ($CDCl_3$) δ 0.97−1.11 (m, 12H), 1.18−1.29 (m, 2H), 3.95 (t, 1H, J = 3.1 Hz), 4.36 (s, 2H), 7.30 (d, 2H, J = 7.8 Hz), 7.54 (d, 2H, J = 8.1 Hz); ^{13}C NMR ($CDCl_3$) δ 10.6, 18.4, 18.6, 54.8, 127.3, 134.4, 135.9, 136.2. Anal. Calcd for $C_{13}H_{21}N_3Si$: C, 63.11; H, 8.45; N, 16.83. Found: C, 63.42; H, 8.45; N, 16.83.

Synthesis of Compound 1a. Compound **5** (742 mg, 3 mmol) was rendered anhydrous by repeated coevaporation with dry CH_3CN (3 mL × 3) and finally dissolved in dry CH_2Cl_2 (15 mL). To the mixture was added 1,3-dichloro-5,5-dimethylhydantoin (1.18 g, 6 mmol). After being stirred at room temperature for 2 h, imidazole (1.02 g, 15 mmol) and 5′-*O*-(4,4′-dimethoxytrityl)thymidine **6a** (1.68 g, 2.7 mmol) were added. After the mixture was stirred at room temperature for 2 h, the mixture was partitioned between $CHCl_3$ (100 mL) and brine (100 mL). The organic phase was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (40 g) with hexane/$CHCl_3$ (50:50−0:100, v/v) containing 1% pyridine and then $CHCl_3$/MeOH (100:0−97:3, v/v) containing 1% pyridine to give **1a** (1.98 g, 93%): ^{1}H NMR ($CDCl_3$) δ 0.94−1.07 (m, 12H), 1.17−1.27 (m, 2H), 1.47 (s, 3H), 2.19−2.29 (m, 1H), 2.41−2.48 (m, 1H), 3.25 (dd, 1H, J = 2.4, 10.5 Hz), 3.47 (dd, 1H, J = 2.3, 10.6 Hz), 3.79 (s, 6H), 4.13 (d, 1H, J = 2.2 Hz), 4.35 (s, 2H), 4.64−4.66 (m, 1H), 6.47 (dd, 1H, J = 5.8, 7.7 Hz), 6.79 (dd, 4H, J = 2.2, 8.9 Hz), 7.23−7.37 (m, 11H), 7.48 (d, 2H, J = 8.1 Hz), 7.65 (s, 1H), 8.32 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 11.8, 11.9,12.0, 12.5, 17.3, 40.6, 41.8, 54.7, 55.2, 62.4,63.3, 72.9, 73.4, 77.2, 81.4, 84.9, 86.9, 87.2, 87.5, 88.0, 111.1, 113.1, 113.2, 127.0, 127.1, 127.4, 127.5, 127.7, 127.8, 127.9, 128.0, 129.1, 130.0, 133.8, 133.9, 134.9, 135.0, 135.3, 135.6, 136.7, 137.1, 139.4, 144.2, 147.3, 150.2, 150.3, 158.6, 158.7, 163.5, 163.7; HRMS (ESI) calcd for $[C_{44}H_{51}N_5O_7Si + Na]^+$ 812.3455, found 812.3461.

Synthesis of Compound 1b. Compound **5** (247 mg, 1 mmol) was rendered anhydrous by repeated coevaporation with dry CH_3CN (3 mL × 3) and finally dissolved in dry CH_2Cl_2 (5 mL). To the mixture was added 1,3-dichloro-5,5-dimethylhydantoin (394 mg, 2 mmol). After being stirred at room temperature for 2 h, imidazole (340 mg, 5 mmol) and 5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyadenosine **6b** (498 mg, 0.9 mmol) were added. After the mixture was stirred at room temperature for 2 h, the mixture was partitioned between $CHCl_3$ (50 mL) and brine (50 mL). The organic phase was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (15 g) with hexane/$CHCl_3$ (50:50−0:100, v/v) containing 1% pyridine and then $CHCl_3$/MeOH (100:0−97:3, v/v) containing 1% pyridine to give **1b** (469 mg, 65%): ^{1}H NMR

($CDCl_3$) δ 0.97−1.05 (m, 12H), 1.25−1.28 (m, 2H), 2.52−2.58 (m, 1H), 2.75−2.80 (m, 1H), 3.29 (dd, 1H, J = 4.4, 10.5 Hz), 3.40 (dd, 1H, J = 4.4, 10.4 Hz), 3.80 (s, 6H), 4.28 (d, 1H, J = 2.4 Hz), 4.35 (s, 2H), 4.73 (t, 1H, J = 2.7 Hz), 6.04 (br s, 2H), 6.50 (t, 1H, J = 6.7 Hz), 6.77 (d, J = 8.8 Hz), 7.20−7.28 (m, 9H), 7.35 (d, 2H, J = 6.8 Hz), 7.51 (d, 2H, J = 7.6 Hz), 8.02 (s, 1H), 8.28 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 12.1, 12.2, 17.5, 41.0, 54.9, 55.3, 63.7, 73.7, 84.7, 86.7, 87.3, 113.3, 120.3, 127.0, 127.6, 128.3, 130.2, 134.1, 135.1, 135.8, 136.9, 139.3, 144.7, 149.8, 152.9, 155.5, 158.7; HRMS (ESI) calcd for $[C_{44}H_{50}N_8O_5Si + H]^+$ 799.3752, found 799.3751.

Synthesis of Compound 7. Compound **5** (65 mg, 0.26 mmol) was rendered anhydrous by repeated coevaporation with dry CH_3CN (3 mL × 3) and finally dissolved in dry CH_2Cl_2 (5 mL). To the mixture was added 1,3-dichloro-5,5-dimethylhydantoin (102 mg, 0.52 mmol). After being stirred at room temperature for 2 h, imidazole (88 mg, 1.3 mmol) and 5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxycytidine **6c** (124 mg, 0.23 mmol) were added. After the mixture was stirred at room temperature for 30 min, the mixture was partitioned between $CHCl_3$ (50 mL) and brine (50 mL). The organic phase was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure The residue was chromatographed on a column of silica gel (10 g) with hexane/$CHCl_3$ (50:50−0:100, v/v) containing 1% pyridine and then $CHCl_3$/MeOH (100:0−97:3, v/v) containing 1% pyridine to give **7** (101 mg, 53%): ^{1}H NMR ($CDCl_3$) δ 0.96−1.07 (m, 12H), 1.20−1.26 (m, 2H), 2.14−2.20 (m, 1H), 2.39−2.44 (m, 1H), 3.32 (d, 1H, J = 7.5 Hz), 3.46 (d, 1H, J = 7.5 Hz), 3.79 (s, 1H), 4.11 (s, 1H), 4.36 (s, 2H), 4.65 (t, 1H, J = 6.3 Hz), 5.49 (d, 1H, J = 8.0 Hz), 6.37 (t, 1H, J = 6.5 Hz), 6.81−6.84 (m, 4H), 7.22−7.50 (m, 14H), 8.43 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 11.9, 12.0, 12.4, 17.3, 41.5, 54.6, 54.7, 55.1, 55.2, 55.3, 62.8, 62.9, 63.0, 72.7, 72.8, 77.2, 84.7, 84.8, 86.8, 86.9, 99.9, 113.1, 113.2, 127.1, 12.3, 127.4, 127.5, 127.9, 128.0, 128.1, 130.0, 130.1, 133.1, 133.7, 134.6, 134.9, 135.0, 135.2, 136.8, 144.1, 148.3, 156.3, 158.6; HRMS (ESI) calcd for $[C_{43}H_{49}ClN_6O_6Si + Na]^+$ 831.3069, found 831.3049.

Synthesis of Compound 9. Compound **8** (456 mg, 1 mmol) was rendered anhydrous by repeated coevaporation with dry CH_3CN (3 mL × 3) and finally dissolved in dry CH_2Cl_2 (5 mL). To the mixture was added 1,3-dichloro-5,5-dimethylhydantoin (216 mg, 1.1 mmol). After the mixture was stirred at room temperature for 10 min, the mixture was partitioned between $CHCl_3$ (50 mL) and brine (50 mL). The organic phase was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (15 g) with hexane/$CHCl_3$ (50:50−0:100, v/v) and then $CHCl_3$/MeOH (100:0−97:3, v/v) to give **9** (455 mg, 93%): ^{1}H NMR ($CDCl_3$) δ 0.00−0.041 (m, 12H), 0.69−0.82 (m, 18H), 1.89−1.99 (m, 1H), 2.11−2.19 (m, 1H), 3.64 (d, 1H, J = 9.5 Hz), 3.75−3.80 (m, 2H), 4.28−4.33 (m, 1H), 5.69 (d, 1H, J = 5.1 Hz), 6.17 (t, 1H, J = 6.3 Hz), 7.34 (d, 1H, J = 5.1 Hz), 8.48 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ −5.7, −5.6, −4.9, −4.7, 17.9, 18.2, 25.6, 25.8, 41.3, 62.4, 71.4, 84.7, 87.6, 99.7, 99.9, 132.9, 133.0, 148.3, 156.3, 156.4; HRMS (ESI) calcd for $[C_{21}H_{40}N_2{}^{15}NO_4Si + H]^+$ 491.2295, found 491.2283.

Synthesis of Compound 1c. Compound **5** (136 mg, 1 mmol) was rendered anhydrous by repeated coevaporation with dry CH_3CN (3 mL × 3) and finally dissolved in dry CH_2Cl_2 (5 mL). To the mixture was added 1,3-dichloro-5,5-dimethylhydantoin (394 mg, 2 mmol). After being stirred at room temperature for 2 h, imidazole (340 mg, 5 mmol) and 5′-*O*-(4,4′-dimethoxytrityl)-*N*^{4}-acetyl-2′-deoxycytidine **6d** (514 mg, 0.9 mmol) were added. After the mixture was stirred at room temperature for 2 h, the mixture was partitioned between $CHCl_3$ (100 mL) and brine (100 mL). The organic phase was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (15 g) with hexane/$CHCl_3$ (50:50−0:100, v/v) containing 1% Et_3N and then $CHCl_3$/MeOH (100:0−97:3, v/v) containing 1% Et_3N to give the fractions containing the desired nucleoside **10** having a Si−O bond. The fractions were collected and evaporated under reduced pressure. The residue was finally evaporated by repeated coevaporation three times each with toluene and $CHCl_3$ to remove the last traces of pyridine. Subsequently, the residue was dissolved in 20% tBuNH$_2$/MeOH (10 mL). After the mixture was stirred at room temperature for 7.5 h, the mixture was evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (15 g) with hexane/$CHCl_3$ (50:50−0:100, v/v) containing 1% pyridine and then $CHCl_3$/MeOH (100:0−97:3, v/v) containing 1% pyridine to give **1c** (564 mg, 81%): ^{1}H NMR ($CDCl_3$) δ 0.90−0.96 (m, 12H), 1.03−1.18 (m, 2H), 2.11−2.19 (m, 1H), 2.56−2.62 (m, 1H), 3.27 (dd, 1H, J = 3.2, 10.5 Hz), 3.52 (dd, 1H, J = 2.7, 10.8 Hz), 3.77 (s, 6H), 4.10 (d, 1H, J = 3.8 Hz), 4.32 (s, 2H), 4.55−4.59 (m, 1H), 5.42 (d, 1H, J = 7.3 Hz), 6.32 (t, 1H, J = 5 0.7 Hz), 6.81 (dd, 4H, J = 2.2, 8.6 Hz), 7.20−7.47 (m, 13H), 7.99 (d, 1H, J = 7.3 Hz); ^{13}C NMR ($CDCl_3$) δ 12.2, 17.5, 42.7, 54.9, 55.4, 62.1, 77.2, 86.4, 86.8, 86.9, 94.0, 113.3, 113.4, 127., 127.6, 128.0, 128.3, 128.4, 130.3, 133.3, 134.0, 135.1, 135.5, 136.8, 141.6, 144.4, 155.9, 158.8, 165.5; HRMS (ESI) calcd for $[C_{43}H_{50}N_8O_6Si + Na]^+$ 797.3459, found 797.3452.

Synthesis of Compound 1d. Compound **5** (136 mg, 0.55 mmol) was rendered anhydrous by repeated coevaporation with dry CH_3CN (3 mL × 3) and finally dissolved in dry CH_2Cl_2 (5 mL). To the mixture was added 1,3-dichloro-5,5-dimethylhydantoin (217 mg, 1.1 mmol). After being stirred at room temperature for 2 h, imidazole (170 mg, 2.5 mmol) and 5′-*O*-(4,4′-dimethoxytrityl)-*N*^{2}-dimethylaminomethylene-2′-deoxyguanosine **6e** (312 mg, 0.5 mmol) were added. After the mixture was stirred at room temperature for 2 h, the mixture was partitioned between $CHCl_3$ (50 mL) and brine (50 mL). The organic phase was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (10 g) with hexane/$CHCl_3$ (50:50−0:100, v/v) containing 1% Et_3N and then $CHCl_3$/MeOH (100:0−97:3, v/v) containing 1% Et_3N to give the fractions containing the desired nucleoside **11** having a Si−O bond. The fractions were collected and evaporated under reduced pressure. The residue was finally evaporated by repeated coevaporation three times each with toluene and $CHCl_3$ to remove the last traces of pyridine. Subsequently, the residue was dissolved in 2 M $MeNH_2$/THF (5 mL). After the mixture was stirred at room temperature for 7.5 h, the mixture was evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (10 g) with hexane/$CHCl_3$ (50:50−0:100, v/v) containing 1% pyridine and then $CHCl_3$/MeOH (100:0−97:3, v/v) containing 1% pyridine to give **1d** (204 mg, 50%): ^{1}H NMR (DMSO-d_6) δ 0.90−0.958 (m, 12H), 1.15−1.24 (m, 2H), 2.34−2.50 (m, 1H), 2.69−2.43 (m, 1H), 3.10−3.25 (m, 2H), 3.71 (s, 6H), 3.98 (s, 1H), 4.46 (s, 2H), 4.59 (s, 1H), 6.19 (d, 1H, J = 5.7 Hz), 6.43 (br s, 2H), 6.81 (d, 4H, J = 7.0 Hz), 7.17−7.32 (m, 11H), 7.48 (d, 1H, J = 5.7 Hz), 7.78 (d, 1H, J = 4.6 Hz), 8.31 (d, 1H, J = 5.9 Hz), 10.63 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 11.1, 11.2, 17.1, 17.2, 24.6, 53.5, 55.0, 62.8, 63.5, 72.9, 79.1, 82.1, 85.7, 85.8, 112.2, 113.1, 116.8, 126.6, 127.6, 127.7, 129.6, 133.1, 134.6, 134.7, 134.8, 135.4, 137.0, 144.7, 144.7, 151.1, 153.7, 156.7, 158.0; HRMS (ESI) calcd for $[C_{44}H_{50}N_8O_6Si + Na]^+$ 837.3520, found 837.3529.

Synthesis of Resin 12. A highly cross-linked polystyrene (HCP) resin (1 g) having a benzylamino group (34 μmol/g) was washed with dry CH_3CN (1 mL × 3) and dried under reduced pressure. The HCP resin was added in dry CH_2Cl_2 (10 mL) in a round flask. To the mixture were added propiolic acid (20 μL, 170 μmol) and DCC (175 mg, 850 μmol). After a round flask having the mixture was attached to a rotary evaporator and gently rotated for 3 h, the solvent was removed by filtration. The introduction of propiolic acid described above was repeated again. The residual CPG was washed by use of CH_2Cl_2 (1 mL × 3) and dried under reduced pressure. Subsequently, the resin was dissolved in pyridine/Ac_2O (9:1, v/v, 10 mL) in a round flask. To the mixture was added 4-(dimethylamino)pyridine (30 mg, 240 μmol). After a round flask having the mixture was attached to a rotary evaporator and gently

rotated for 2 h, the solvent was removed by filtration to give the HCP resin **12**.

Synthesis of 3′-Terminal Nucleoside-Loaded Resins 13a−d. The HCP resin **10** (118 mg) with was washed with dry CH_3CN (1 mL × 3) and dried under reduced pressure. The HCP resin was added in dry THF (2 mL) in a round flask. To the mixture were added 3′-terminal nucleoside-loading reagent **1** (40 μmol), Et_3N (5.52 μL, 40 μmol), and CuI (7.6 mg, 40 μmol). After being refluxed for 12 h, the solvent was removed by filtration. The residual HCP resin was washed by use of CH_3CN (1 mL × 3) and dried under reduced pressure. The amount of the nucleoside introduced into the resin was estimated by use of the DMTr cation assay, as shown in Scheme 5.

Synthesis of Oligonucleotides 15a−d and 16. The synthesis of oligodeoxynucleotides was carried out on a HCP resin **13** having a silyl linker in an ABI 392 DNA synthesizer by use of the reaction cycle in activated phosphite method. The 2-cyanoethyl groups of oligomer obtained after chain elongation was deprotected by treatment with a 10% DBU solution in CH_3CN (500 μL) at room temperature for 1 min. Then, the mixture containing oligo DNAs was released from the resin by treatment with a solution of TBAF (1 M) and AcOH (1 M) in THF (500 μL) at room temperature for 24 h. The polymer support was removed by filtration and washed with 0.1 M ammonium acetate buffer (1 mL × 3). The filtrate was purified by anion-exchange HPLC to give oligonucleotides **15a−d** and **16**. Oligonucleotide **15a**: d[TACCTAAATCCAT] MALDI-TOF mass calcd for $[C_{126}H_{161}N_{45}O_{75}P_{12} + H]^+$ 3876.71, found 3874.87. Oligonucleotide **15b**: d[TACCTAAATCCAA] MALDI-TOF mass calcd for $[C_{126}H_{160}N_{48}O_{73}P_{12} + H]^+$ 3885.72, found 3882.48. Oligonucleotide **15c**: d[TACCTAAATCCAC] MALDI-TOF mass calcd for $[C_{125}H_{160}N_{46}O_{74}P_{12} + H]^+$ 3861.71, found 3866.52. Oligonucleotide **15d**: d[TACCTAAATCCAG] MALDI-TOF mass calcd for $[C_{126}H_{160}N_{48}O_{73}P_{12} + H]^+$ 3901.72, found 3904.40. Oligonucleotide **16**: d[A*C*T*C*C*GT*C*T*A*C*G], A* = 8-aza-7-deaza-A, C* = 4-*N*-acetyl-C, T* = 2-thio-T, MALDI-TOF mass calcd for $[C_{129}H_{163}N_{41}O_{75}P_{11}S_3 + H]^+$ 3922.64, found 3921.70.

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Supporting Information Available: The ^1H, ^{13}C data of all new products. This material is available free of charge via the Internet at http://pubs.acs.org.

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