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Efficient synthesis of functionalized oligodeoxyribonucleotides with base-labile groups using a new silyl linker

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Abstract—In this study, we developed new 3′-terminal deoxyribonucleoside-loading reagents **1** with a new silyl-type linker. These reagents could increase the efficiency of introduction of 3′-terminal deoxyribonucleoside components into polymer supports to a level of 17–29 μmol/g. The efficiency was higher than that of previous T-loading reagents because reagents **1** contain a 4-aminobutyryl residue as a spacer. Moreover, we could synthesize not only unmodified DNA oligomers but also a base-labile modified DNA oligomer using resins **9a–d** in the activated phosphite method without base protection.

1. Introduction

DNA and RNA oligonucleotides have been used for the suppression of specific genes,[1–5] the exhaustive analysis of a gene expression,[6,7] and the detection of single nucleotide polymorphisms (SNPs)[8–14] because they can accurately recognize complementary oligonucleotides. A number of modified oligonucleotides containing functional groups have been developed to enhance their effects. For example, 2′-*O*-modified RNA oligomers[15–21] acquired nuclease resistance compared to unmodified ones, and oligomers with modified nucleobases[22–25] have demonstrated increased accuracy of base recognition. Among them, some modified oligomers with base-labile functional groups,[26–28] such as acyl groups. However, such oligomers cannot be obtained if the standard phosphoramidite approach[29] is employed in solid-phase synthesis, because base-labile functional groups are easily decomposed by treatment with concd NH_3 aq for the removal of base protecting groups and for their release from polymer supports.

We have recently developed the 'activated phosphite method' without base protection in phosphoramidite chemistry to efficiently synthesize oligonucleotides containing base-labile functional groups.[30,31] In the activated phosphite method, we can omit the deprotection step for the removal of the protecting groups of nucleobases. If the oligonucleotides are released from polymer supports under neutral conditions without using concd NH_3 aq in the final step of the activated phosphite method, we should overcome the above-mentioned problem.

Kobori et al. previously proposed a loading reagent of a silyl linker[32] with a thymidine instead of a widely useful linker such as a succinyl linker. This linker can be rapidly cleaved by treatment with tetrabutylammonium fluoride (TBAF)–AcOH in THF under neutral conditions. However, the loading reaction using the proposed reagent requires a long time because the reactivity of the reagent is much lower than that of a succinyl linker. The loading amount of thymidine introduced into highly cross-linked polystyrene (HCP) resins,[33] which could increase the coupling efficiency compared to controlled pore glass (CPG) resins in the activated phosphite method was only 6–8 μmol/g for 1 day. This low reactivity might result from the skeleton of the benzoic acid.

In this paper, we report new loading reagents **1a–d** (Fig. 1) with a silyl linker and four kinds of 2′-deoxynucleosides to increase the reactivity of the reagent and the synthesis of DNA oligomers using this silyl linker in the activated phosphite method.

Keywords: DNA synthesis; Silyl linker; N-unprotected phosphoramidite approach; Solid-phase synthesis.
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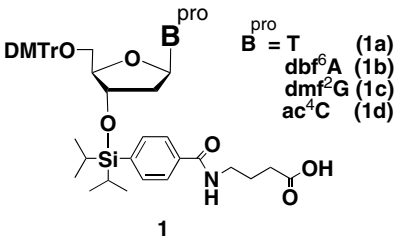
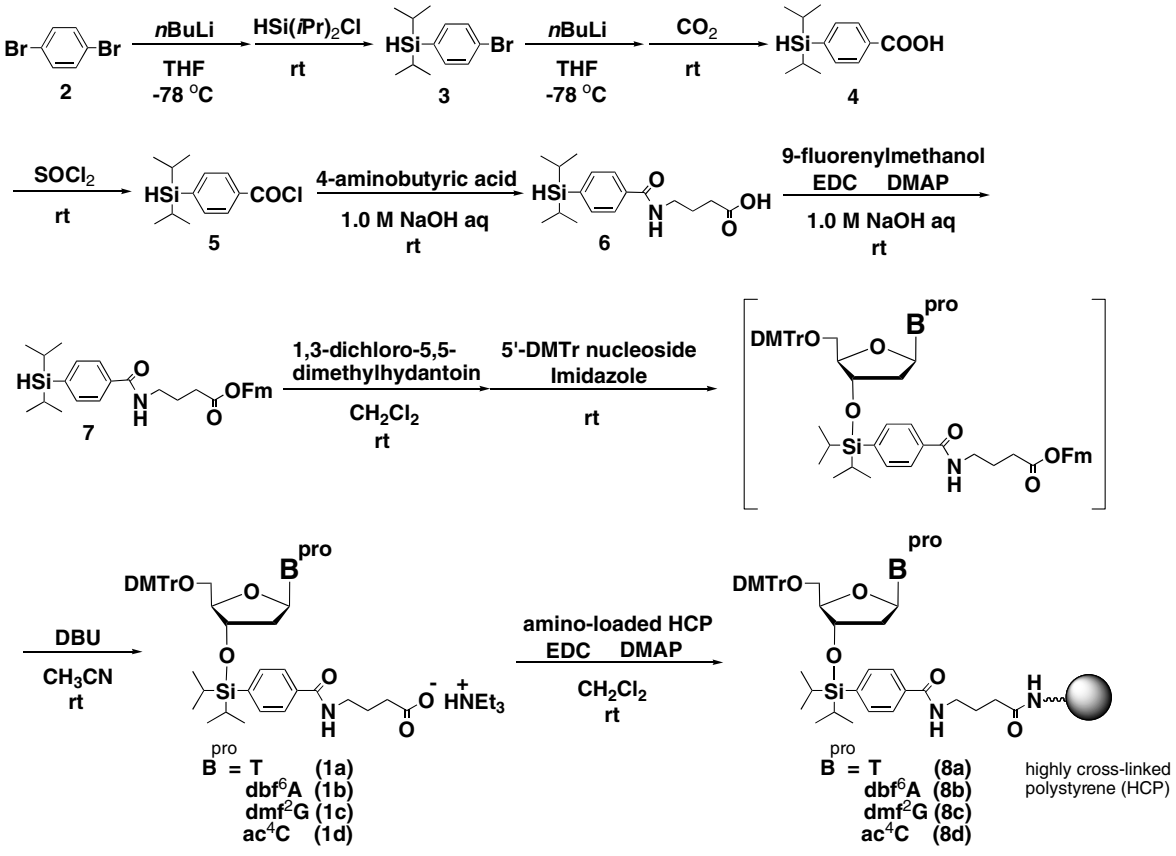


Figure 1. Skeleton of loading reagents containing a silyl linker.

2. Result and discussion

To increase the loading amount of a nucleoside into HCP resins, we designed new loading reagents **1** containing a silyl linker that has a 4-aminobutyric acid residue as a spacer. Scheme 1 shows an efficient synthetic route for T-, A-, G-, or C-loaded HCP resins containing a silyl linker. 1,4-dibromobenzene **2** was carried out using stepwise halogen–metal exchange reactions to obtain compound **4** according to the previous method.[34] Compound **4** was converted to benzoyl chloride derivative **5** by treatment with $SOCl_2$ in 77% yield. Subsequently, the reaction of compound **5** with 4-aminobutylic acid in the presence of NaOH gave compound **6** in 65% yield. The carboxyl group of compound **6** was protected by the 9-fluorenylmethyl (Fm) group using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) as a condensing agent to give compound **7**. The isolated yield of compound **7** by purification using silica-gel column chromatography was 80%. Compound **7** was converted to a silyl chloride derivative by treatment with 1,3-dichloro-5,5-dimethylhydantoin. The in situ generated silyl chloride was allowed to react with 5′-*O*-DMTr nucleosides (T, A, G and C) in the presence of imidazole at room temperature for 30 min. The amino groups of these 5′-*O*-DMTr nucleosides were protected with *N*,*N*-dibutylaminomethylene (dbf), *N*,*N*-dimethylaminomethylene (dmf), or acetyl groups to avoid side reactions with nucleobases during the introduction of the silyl linker into the HCP resin. For the synthesis of the loading reagent **1**, the selective deprotection of the Fm groups was carried out by treatment with DBU in CH_3CN at room temperature for 10 min. The reagents **1a**–**d** were obtained from compound **7** via two steps using silica-gel column chromatography with 1% Et_3N in 65%, 60%, 53%, and 68% yields, respectively. Reagents **1a**–**d** were condensed with the amino groups of the HCP resin using DCC in CH_2Cl_2 for 12 h. Finally, the capping reactions of these resins by treatment with Ac_2O and DMAP in pyridine were carried out with the desired T-, dA-, dG-, and dC-loaded HCP resins **8**. The loading amounts of nucleosides were estimated by DMTr cation assay as 27, 26, 31, and 21 μmol/g, respectively. For the first time, we could introduce four kinds of 2′-deoxyribonucleosides bridged by a silyl linker to polymer supports.

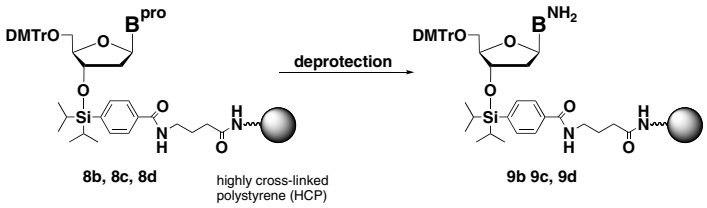
The protecting group should be removed at the first step of DNA synthesis to avoid decomposition of a base-labile functional group at the deprotection step when the



Scheme 1. Synthesis of the nucleoside-loaded HCP resins **8** containing the silyl linker.

nucleoside-loaded HCP resins **8** were used in the activated phosphite method without base protection. Therefore, we selected a non-aqueous treatment, $MeNH_2$/THF,[34] to remove the protecting groups of nucleobases without eliminating the nucleoside from the HCP resin. First, the T-loaded HCP was treated with $MeNH_2$/THF for 6 h to estimate the stability of the Si–O bond. As a result, the ratio of elimination of the nucleoside residue from the resin was found to be less than 3%, estimated by the DMTr cation assay. This result indicates that the Si–O bond in the T-loaded HCP is stable under the deprotection conditions. Subsequently, the deprotection of dbf^{6}A-loaded HCP was carried out by treatment with $MeNH_2$/THF, as shown in Figure 2. The HPLC analysis shows that the dbf group of the adenine was completely cleaved in over 3 h. Unexpectedly, the elimination of the nucleoside residue from the resin increased to 8%. These results indicate that the stability of the Si–O bond depends on the kind of nucleobase present.[35] In the case of guanine, 6% elimination of the nucleoside from the resin was observed during the deprotection when the dmf group could be rapidly cleaved by treatment with $MeNH_2$/THF. The elimination of cytosine surprisingly increased to a level of 25% in 3.5 h under the same conditions. To suppress the elimination of dC from the resin, the deprotection of the acetyl group was carried out by treatment with 2 M NH_3/MeOH instead of $MeNH_2$/THF. Because the nucleophilicity of NH_3 is lower than that of $MeNH_2$, we could decrease the elimination to 18% though the reaction rate of the deprotection was slower than that by treatment with 2 M $MeNH_2$/THF. Consequently, for the first time, we could synthesize dA-, dG-, and dC-loaded HCP resins **9** containing a silyl linker without base protection. The loading amounts of nucleosides were finally estimated by the DMTr cation assay as 24, 29, and 17 μmol/g, respectively.

Previously, we showed the synthesis of oligo DNAs with base-labile functional groups using T-loaded HCP resin **8a**. The oligo DNAs could quantitatively be released without their decomposition by treatment with TBAF–AcOH or $Et_3N{\cdot}3HF$ under neutral conditions. In this paper, the first synthesis of oligodeoxynucleotides using A-, G-, or C-loaded HCP resins (**9b–d**) containing the silyl linker is reported. We demonstrated the synthesis of three unmodified DNA oligomers using the activated phosphite method and a silyl linker, as shown in Scheme 2. Each chain elongation was carried out using 6-nitro-1-hydroxybenzotiazole[36] in the presence of benzimidazolium triflate (BIT)[37] as an activator on the A-, G-, or C-loaded HCP resins. After chain elongation, the selective removal of the cyanoethyl groups of the internucleotidic phosphates was carried out by treatment with 1 M DBU in CH_3CN for 1 min and the successive release of the 5′-terminal DMTr group was carried out by treatment with 3% trichloroacetic acid in CH_2Cl_2. Finally, the unmodified DNA oligomers **10–12** were released from the resin by treatment with 0.2 M $Et_3N{\cdot}3HF$ in THF at room temperature for 4 h. The crude products were checked by anion-exchange HPLC, as shown in Figure 3. Purification gave the oligomers **10–12** in 38%, 33%, and 41% yields, respectively. These oligomers were characterized by MALDI-TOF mass spectroscopy. These results show that the A-, G-, and C-loaded HCP resins containing a silyl linker are very useful for the synthesis of unmodified DNA oligomers with the activated phosphite method. Furthermore, we tried to synthesize a base-labile modified DNA 10mer d[CCACGA*GTGG] **13** containing 6-acetyl-8-aza-7-deaza-2′-deoxyadenosine (A* = ac^{6}az^{8}c^{7}A).[38] In our recent study, it turned out that introduction of the modified base, ac^{6}az^{8}c^{7}A, into DNA oligomers increased the hybridization affinity for complementary DNA oligomers and their base recognition ability compared to unmodified adenine base. However, significant elimination of the acetyl group occurred by treatment with concd NH_3 aq for deprotection and release of the DNA oligomer in a standard procedure of DNA synthesis. Therefore, we carried out the synthesis of the modified DNA **13** with the activated phosphite method using the G-loaded HCP resin **9c** with a silyl linker. As a result, the modified DNA **13** could be synthesized without their decomposition and oligomer **13** was obtained in 22% yield, as shown in Figure 3d. These oligomers were characterized by MALDI-TOF mass spectroscopy.

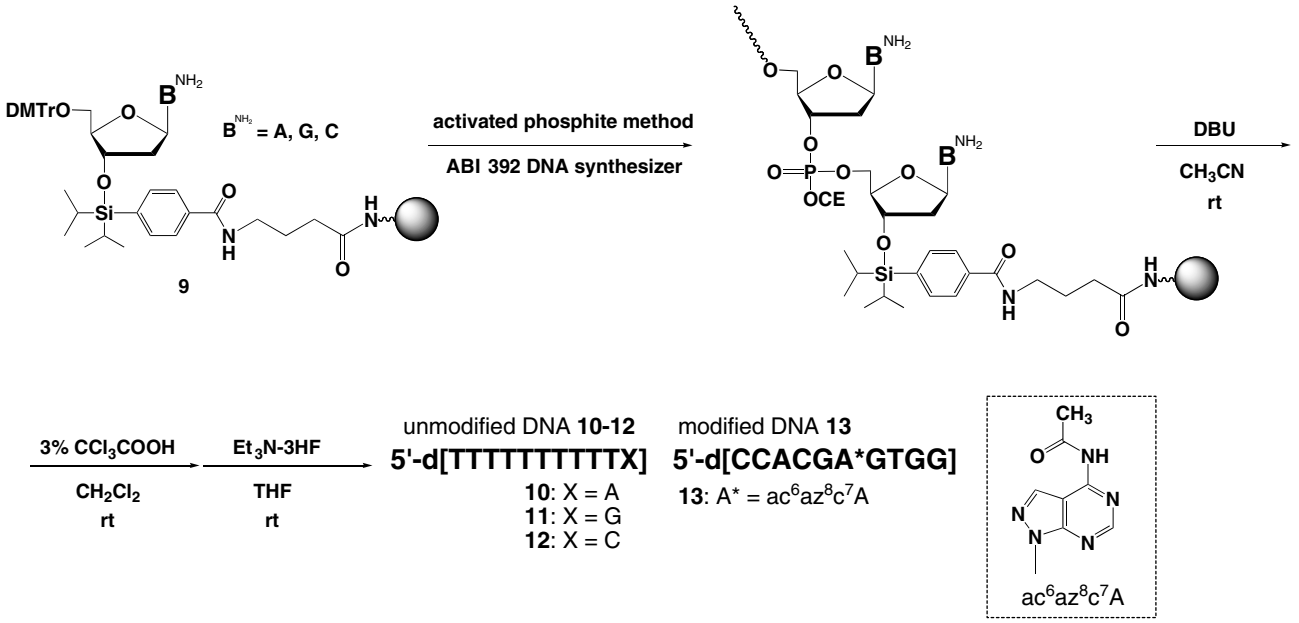


entry	nucleobase	conditions	Tcomp[a]	elimination of nucleoside from the HCP resin[b]
1	dbf^{6}A	2 M $MeNH_2$/THF	3 h	8%
2	dmf^{2}G	2 M $MeNH_2$/THF	0.5 h	6%
3	ac^{4}C	2 M $MeNH_2$/THF	3.5 h	25%
4	ac^{4}C	2 M NH_3/MeOH	15 h	18%

[a] Tcomp was analyzed by using HPLC.

[b] Elimination of nucleoside was analyzed by using DMTr cation assay.

Figure 2. Removal of the protecting groups on the amino groups of nucleobases on HCP resins.



Scheme 2. Synthesis of unmodified DNA **10**–**12** and modified DNA **13**.

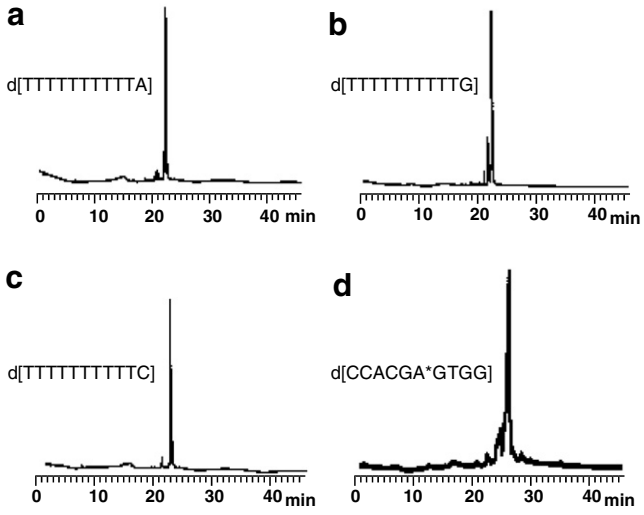


Figure 3. Anion-exchange HPLC profiles of the crude mixtures obtained using the activated phosphite method under the conditions used for ABI 392 DNA synthesizer. (a) d[TTTTTTTTTA], (b) d[TTTTTTTTTG], (c) d[TTTTTTTTTC], and (d) d[CCACGA*GTGG], A* = $ac^6az^8c^7A$.

3. Conclusions

For the first time, we have synthesized the loading reagents **1a**–**d** with a silyl-type of linker and carried out efficient introduction of them into HCP resins **8a**–**d**. Subsequently, the deprotection of nucleobase on their resins was removed by treatment with $MeNH_2$/THF or NH_3/MeOH to prepare resins **9b**–**d**. Moreover, we could synthesize not only unmodified DNA oligomers but also a base-labile modified DNA oligomer using resins **9b**–**d** with the activated phosphite method. The use of a silyl linker in the activated phosphite method has prompted us to study the synthesis of base-labile DNAs such as oxidatively damaged DNAs or, in the near future, functional DNAs and RNAs with various base-labile modified structures. Further studies in this direction are underway.

4. Experimental

4.1. General remarks

^1H, ^{13}C, and ^{31}P NMR spectra were recorded at 270, 68 and 109 MHz, respectively. The chemical shifts were measured from tetramethylsilane for ^1H NMR spectra, $CDCl_3$ (77 ppm) for ^{13}C NMR spectra and 85% phosphoric acid (0 ppm) for ^{31}P NMR spectra. UV spectra were recorded on a U-2000 spectrometer. Column chromatography was performed with silica-gel C-200 purchased from Wako Co. Ltd, and a minipump for a goldfish bowl was conveniently used to attain sufficient pressure for rapid chromatographic separation. Anion-exchange HPLC was done on a Waters Aliance system with a Waters 3D UV detector and a Gen-PakTM FAX column (Waters, 4.6 × 100 mm). A linear gradient (10–60%) of solvent I (1 M NaCl in 25 mM phosphate buffer (pH 6.0)) in solvent II (25 mM phosphate buffer (pH 6.0)) was used at 50 °C at a flow rate of 1.0 mL/min for 40 min. ESI mass was performed by use of Mariner™ (PerSeptive Biosystems Inc.). MALDI-TOF mass was performed by use of Bruker Daltonics [Matrix: 3-hydoroxypicolinic acid (100 mg/ml) in H_2O–diammonium hydrogen citrate (100 mg/ml) in H_2O (10:1, v/v)]. Highly cross-linked polystyrene was purchased from ABI.

4.1.1. Synthesis of compound 5. Compound **4** (6.7 g, 28.4 mmol) was added to thionyl chloride (3.2 mL, 42.6 mmol). After being refluxed with stirring for 2 h, the mixture was distilled under reduced pressure to give

5 (1.7 g, 91%). Bp 102–104 °C (1 mmHg). ^{1}H NMR ($CDCl_3$) δ 0.96–1.07 (m, 12H), 1.18–1.23 (m, 2H), 3.92 (t, 1H, J = 3.1 Hz), 7.36 (d, 2H, J = 6.2 Hz), 7.48 (d, 2H, J = 6.2 Hz). ^{13}C NMR ($CDCl_3$) δ 10.7, 18.5, 18.7, 123.9, 130.8, 132.9, 136.9. Anal. Calcd for $C_{13}H_{19}ClOSi$: C, 61.27; H, 7.52; Cl, 13.91. Found: C, 61.17; H, 7.48; Cl, 14.14.

4.1.2. Synthesis of compound 6. 4-Aminobutyric acid (910 mg, 8.9 mmol) was dissolved in 1 M NaOH aq (9 mL). To the mixture was added compound **5** (1.7 g, 6.7 mmol). After the mixture was stirred at room temperature for 8 h, 12 M HCl aq was added to the mixture for pH 2. Then, the mixture was partitioned between CH_2Cl_2 (400 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 (30 g) with $CHCl_3$–MeOH (100:0–97:3, v/v) to give **6** (1.4 g, 65%). ^{1}H NMR ($CDCl_3$) δ 0.97–1.08 (m, 12H), 1.25–1.30 (m, 2H), 3.98 (t, 1H, J = 3.1 Hz), 7.63 (d, 2H, J = 7.8 Hz), 7.48 (d, 2H, J = 7.8 Hz). ^{13}C NMR ($CDCl_3$) δ 10.7, 18.5, 18.7, 128.8, 129.7, 135.4, 142.0, 172.4. Anal. Calcd for $C_{17}H_{27}NO_3Si$: C, 63.51; H, 8.47; N, 4.36. Found: C, 63.34; H, 8.43; N, 4.31.

4.1.3. Synthesis of compound 7. Compound **6** (12.5 g, 38.9 mmol) was dissolved in dry CH_2Cl_2 (200 mL). To the mixture were added 9-fluorenylmethanol (11.4 g, 58.4 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (14.8 g, 79.8 mmol), and 4-dimethylaminopyridine (195 mg, 1.6 mmol). After being stirred at room temperature for 3 h, the mixture was partitioned between $CHCl_3$ (300 mL) and brine (500 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 (200 g) with hexane–ethyl acetate (100:0–50:50, v/v) to give **7** (15.5 g, 80%). ^{1}H NMR ($CDCl_3$) δ 0.91–1.02 (m, 12H), 1.14–1.22 (m, 2H), 1.86 (t, 2H, J = 6.8 Hz), 2.41 (t, 2H, J = 7.0 Hz), 3.36 (dd, 2H, J = 6.5 Hz, J = 12.7 Hz), 3.92 (t, 1H, J = 2.7 Hz), 4.36 (d, 2H, J = 7.0 Hz), 6.67 (br s, 1H), 7.21–7.36 (m, 4H), 7.50 (d, 2H, J = 7.0 Hz), 7.68 (d, 2H, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$) δ 10.4, 18.2, 18.4, 24.3, 31.96, 39.2, 46.6, 66.1, 119.8, 124.7, 125.7, 127.0, 127.6, 134.8, 135.4, 141.1, 143.5, 167.5, 173.4, Anal. Calcd for $C_{31}H_{37}NO_3Si$: C, 74.51; H, 7.46; N, 2.80. Found: C, 74.29; H, 7.62; N, 2.86.

4.1.4. A general procedure for the synthesis of compound 1a–d. Compound **7** (830 mg, 1.7 mmol) was rendered anhydrous by repeated coevaporation with dry CH_3CN (3× 3 mL) and finally dissolved in dry CH_2Cl_2 (20 mL). To the mixture was added 1,3-dichloro-5,5-dimethylhydantoin (655 mg, 3.3 mmol). After being stirred at room temperature for 30 min, imidazole (510 mg, 7.5 mmol) and 5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxynucleoside **8** (1.5 mmol) were added. After the mixture was stirred at room temperature for 30 min, 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 (30 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 the fractions containing the desired nucleoside **9** 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 dry CH_3CN (20 mL). To the mixture was added 1,8-diazabicyclo[5.4.0]-7-undecene (670 μL, 4.5 mmol). After the mixture was stirred at room temperature for 10 min, a 0.2 M solution of triethylamine hydrogen carbonte (50 mL) was added to the mixture. After being stirred at room temperature for 5 min, the mixture was partitioned between $CHCl_3$ (100 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 (30 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 **1**. 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 Et_3N to give **1**.

4.1.5. Compound 1a. ^{1}H NMR ($CDCl_3$) δ 0.83–0.95 (m, 12H), 1.04–1.20 (m, 11H), 1.41 (s, 3H), 1.86–1.93 (m, 2H), 2.10–2.17 (m, 1H), 2.28–2.37 (m, 3H), 2.96 (dd, 6H, J = 7.3 Hz, J = 14.6 Hz), 3.21 (d, 1H, J = 8.6 Hz), 3.38–3.41 (m, 3H), 3.70 (s, 6H), 4.07 (s, 1H), 4.57 (s, 1H), 6.37 (t, 1H, J = 5.4 Hz), 6.73 (d, J = 6.8 Hz), 7.17–7.30 (m, 9H), 7.44 (d, 2H, J = 7.6 Hz), 7.57 (s, 1H) 7.74 (d, 2H, J = 7.8 Hz), 7.89 (br s, 1H). ^{13}C NMR ($CDCl_3$) δ 8.3, 11.8, 17.1, 17.2, 17.3, 24.4, 34.2, 40.5, 41.5, 45.1, 55.1, 63.3, 73.6, 77.2, 84.8, 86.9, 87.1, 111.1, 113.1, 126.2, 127.0, 127.9, 129.9, 134.2, 135.11, 135.14, 135.5, 137.4, 144.1, 150.5, 158.6, 164.2, 167.4, 179.2. HRMS (ESI) calcd for $[C_{48}H_{57}N_3O_{10}Si+Na]^+$ 886.3711. Found: 886.37130.

4.1.6. Compound 1b. ^{1}H NMR ($CDCl_3$) δ 0.92–1.02 (m, 18H), 1.12–1.28 (m, 8H), 1.37 (t, 4H, J = 7.3 Hz), 1.65 (dd, 4H, J = 7.2 Hz, J = 14.6 Hz), 1.96 (br s, 2H), 2.43–2.56 (m, 3H), 2.75–2.83 (m, 1H), 2.93 (dd, 4H, J = 7.2 Hz, J = 14.4 Hz), 3.27–3.51 (m, 6H), 3.67–3.76 (m, 8H), 4.24 (d, 1H, J = 2.7 Hz), 4.76 (s, 1H), 6.53 (t, 1H, J = 6.3 Hz), 6.76 (d, 4H, J = 8.4 Hz), 7.20–7.37 (m, 9H), 7.53 (d, 2H, J = 7.8 Hz), 7.82 (d, 2H, J = 7.8 Hz), 8.06 (s, 1H), 8.10 (br s, 1H), 8.50 (s, 1H), 8.96 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 8.5, 11.9, 12.0, 13.6, 13.8, 17.3, 19.7, 20.1, 24.0, 29.1, 30.9, 34.8, 40.6, 41.1, 44.7, 45.1, 51.8, 55.1, 63.2, 73.3, 84.0, 86.4, 86.7, 113.0, 126.2, 126.7, 127.7, 128.0, 129.9, 134.3, 135.6, 135.9, 137.2, 139.8, 144.4, 151.2, 152.6, 158.0, 158.4, 159.9, 167.2, 179.5. HRMS (ESI) *m/z* calcd for $[C_{57}H_{73}N_7O_8Si+H]^+$, 1012.5368. Found: 1012.5371.

4.1.7. Compound 1c. ^{1}H NMR ($CDCl_3$) δ 0.88–1.03 (m, 12H), 1.08–1.20 (m, 11H), 1.90–2.01 (m, 2H), 2.45 (dd, 4H, J = 6.5 Hz, J = 11.6 Hz), 2.93 (dd, 4H, J = 7.3 Hz,

J = 14.0 Hz), 3.09 (2s, 6H), 3.30 (d, J = 4.1 Hz), 3.49 (d, J = 5.7 Hz), 3.77 (s, 6H), 4.23 (s, 1H), 4.62 (s, 1H), 6.42 (t, 1H, J = 6.7 Hz), 6.79 (d, 4H, J = 8.6 Hz), 7.16–7.29 (m, 7H), 7.37 (d, 2H, J = 7.0 Hz), 7.52 (d, 2H, J = 7.6 Hz), 7.71 (s, 1H), 7.83 (d, 2H, J = 7.6 Hz), 8.41 (br s, 1H), 8.55 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 8.8, 11.8, 11.9, 17.17, 17.22, 24.3, 35.1, 35.3, 41.0, 41.3, 41.4, 44.9, 55.1, 63.4, 73.4, 77.2, 83.1, 86.5, 86.8, 113.1, 120.2, 126.8, 127.8, 127.9, 129.9, 134.2, 135.4, 135.6, 135.9, 137.1, 144.3, 150.1, 156.9, 158.0, 158.2, 158.4, 167.1, 180.2. HRMS (ESI) calcd for $[C_{51}H_{61}N_7O_9Si+Na]^+$966.4197. Found: 966.4195.

4.1.8. Compound 1d. ^{1}H NMR ($CDCl_3$) δ 0.84–0.91 (m, 12H), 1.05–1.15 (m, 5H), 1.87–2.04 (m, 3H), 2.15 (s, 3H), 2.38 (t, 2H, J = 6.3 Hz), 2.50–2.63 (m, 1H), 2.93 (dd, 4H, J = 7.2 Hz, J = 14.4 Hz), 3.23–3.47 (m, 4H), 3.71 (s, 6H), 4.13 (d, 1H, J = 2.7 Hz), 4.45 (d, 1H, J = 4.1 Hz), 6.19 (t, 1H, J = 5.7 Hz), 6.73 (d, 4H, J = 8.1 Hz), 7.08–7.27 (m, 9H), 7.42 (d, 2H, J = 7.8 Hz), 7.54 (br s, 1H), 7.70 (d, 2H, J = 7.8 Hz), 8.18 (d, 1H, J = 7.3 Hz); ^{13}C NMR ($CDCl_3$) δ 7.8, 8.2, 11.7, 17.0, 17.1, 24.3, 24.5, 33.5, 40.1, 42.3, 44.8, 55.0, 57.8, 62.2, 71.9, 77.2, 86.7, 87.0, 96.4, 113.0, 126.0, 126.9, 127.7, 127.8, 129.8, 134.2, 134.8, 135.4, 137.2, 143.8, 144.3, 154.7, 158.4, 162.5, 167.3, 170.8, 178.7. HRMS (ESI) calcd for $[C_{49}H_{58}N_4O_{10}Si+Na]^+$ 913.3820. Found: 913.3822.

4.1.9. A general procedure for the synthesis of 8. A highly cross-linked polystyrene (HCP) resin (1 g) having a benzylamino group was washed with dry CH_3CN (3× 1 mL) and dried under reduced pressure. The HCP resin was added in dry CH_2Cl_2 (3 mL) in a round flask. To the mixture were added compound **1** (168 μmol) and DCC (174 mg, 842 μmol). After a round flask having the mixture was attached to a rotary evaporator and gently rotated for 12 h, the solvent was removed by filtration. The residual CPG was washed by use of CH_2Cl_2 (3× 1 mL) 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. The residual HCP resin **8** was washed with CH_3CN (3× 1 mL) and dried under reduced pressure. The amount of the nucleoside introduced into the resin was estimated by use of the DMTr cation assay.

4.1.10. Synthesis of compounds 9b–d. Removal of the protecting groups on the HCP resin **8b**, **8c**, or **8d** was carried out by treatment with $MeNH_2$/THF or NH_3/MeOH (500 μL). The T_{comp} of the reactions were analyzed by use of DMTr cation assay. The ratio of the eliminated nucleosides from HCP resins was estimated by use of the DMTr cation assay, as shown in Figure 2.

4.1.11. Synthesis of oligonucleotides 10–13. The synthesis of oligodeoxynucleotides **10**–**13** was carried out on a HCP resin **9** having a silyl linker in an ABI 392 DNA synthesizer by use of the reaction cycle in activated phosphite method.[31] 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 $Et_3N{\cdot}3HF$ (0.2 M) and Et_3N (0.4 M) in THF (500 μL) at room temperature for 4 h. The polymer support was removed by filtration and washed with 0.1 M ammonium acetate buffer (3× 1 mL). The filtrate was purified by anion-exchange HPLC to give oligonucleotides **10**–**13**.

*Oligonucleotide **10***. TTTTTTTTTTA MALDI-TOF Mass calcd for $[C_{110}H_{143}N_{25}O_{73}P_{10}+H]^+$ 3294.18. Found: 3293.53.

*Oligonucleotide **11***. TTTTTTTTTTG MALDI-TOF Mass calcd for $[C_{110}H_{143}N_{25}O_{74}P_{10}+H]^+$ 3310.18. Found: 3311.64.

*Oligonucleotide **12***. TTTTTTTTTTC MALDI-TOF Mass calcd for $[C_{109}H_{143}N_{23}O_{74}P_{10}+H]^+$ 3270.15. Found: 3270.72.

*Oligonucleotide **13***. CCACGA*GTGG (A*: $ac^6az^8c^7A$) MALDI-TOF Mass calcd for $[C_{99}H_{124}N_{41}O_{58}P_9+H]^+$ 3096.05. Found: 3094.51.

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