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Synthesis and Properties of Nucleic Acid Analogues Consisting of a Benzene−Phosphate Backbone

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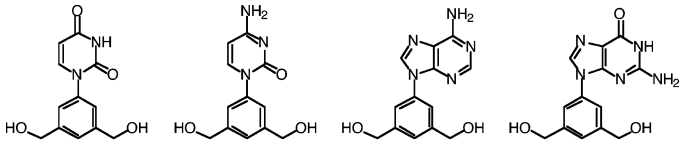

The synthesis and properties of a nucleic acid analogue consisting of a benzene−phosphate backbone are described. The building blocks of the nucleic acid analogue are composed of bis(hydroxymethyl)-benzene residues connected to nucleobases via the biaryl-like axis. Stabilities of the duplexes were studied by thermal denaturation. It was found that the thermal stabilities of the duplexes composed of the benzene−phosphate backbone are highly dependent on their sequences. The duplexes with the benzene−phosphate backbone comprised of the mixed sequences were thermally less stable than the natural DNA duplexes, whereas that composed of the homopyrimidine and homopurine sequences was thermally and thermodynamically more stable than the corresponding natural DNA duplex. It was suggested that the analogues more efficiently stabilize the duplexes in a B-form duplex rather than in an A-form duplex. Thus, the duplexes consisting of the benzene−phosphate backbone, especially composed of the homopyrimidine and homopurine sequences, may offer a novel structural motif useful for developing novel materials applicable in the fields of bio- and nanotechnologies.

Introduction

One major goal of biotechnology and nanotechnology is the assembly of novel biomaterials that can be used for analytical, industrial, and therapeutic purposes. So far, a wide variety of oligonucleotide analogues (ONAs) have been synthesized, and their properties have been extensively investigated in relation to such purposes. For instance, ONAs consisting of six-membered sugar units instead of a natural nucleoside have been synthesized,[1] and their properties have been intensively studied by Eschenmoser's and Herdewijn's groups.[2] Peptide nucleic acids (PNAs) composed of a (2-aminoethyl)glycine backbone developed by Nielsen's group were shown to form thermally stable duplexes with a complementary DNA or RNA,[3,4] and have been applied to antisense studies.

On the other hand, it is known that biaryl compounds have various interplanar angles depending on the substituents on their aromatic rings.[5] Recently, Sauter and Leumann reported the synthesis of an ONA consisting of a building block composed of a uracil base attached to a thiophene residue via the biaryl-like axis.[6,7] The ONA is expected to possess a novel structure and properties

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(1) For examples: (a) Zhang, Y.; Seeman, N. C. *J. Am. Chem. Soc.* **1994**, *116*, 1661−1669. (b) Trotta, P. P.; Beutel, B. A.; Sherman, M. I. *Med. Res. Rev.* **1995**, *15*, 277−298. (c) Praseuth, D.; Guieysse, A. L.; Hélène, C. *Biochim. Biophys. Acta* **1999**, *1498*, 181−206. (d) Leumann, C. J. *Bioorg. Med. Chem.* **2002**, *10*, 841−854.

(2) For examples: (a) Müller, D.; Pitsch, S.; Kittaka, A.; Wagner, E.; Winter, C. E.; Eschenmoser, A. *Helv. Chim. Acta* **1990**, *73*, 1410−1468. (b) Krishnamurthy, R.; Pitsch, S.; Minton, M.; Miculka, C.; Windhab, N.; Eschenmoser, A. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1537−1541. (c) Schöning, K.-U.; Scholz, P.; Guntha, S.; Wu, X.; Krishnamurthy, R.; Eschenmoser, A. *Science* **2000**, *290*, 1347−1351. (d) Herdewijn, P. *Angew. Chem. Int. Ed.* **2001**, *40*, 2249−2251. (e) Lescrinier, E.; Froeyen, M.; Herdewijn, P. *Nucleic Acids Res.* **2003**, *31*, 2975−2989.

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(6) Sauter, G.; Stulz, E.; Leumann, C. *Helv. Chim. Acta* **1998**, *81*, 14−34.

(7) Sauter, G.; Leumann, C. *Helv. Chim. Acta* **1998**, *81*, 916−931.

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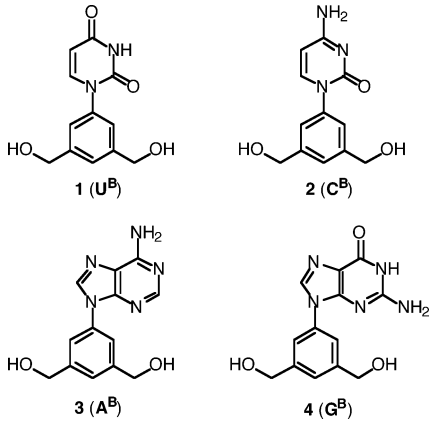

FIGURE 1. Structures of nucleoside analogues.

different from those of a natural ON, since it has a π-electron-rich backbone instead of a natural ribose−phosphate backbone. They attempted to synthesize a homo-hexamer consisting of the uridine analogue but have not yet succeeded. It was revealed that an unwelcome side reaction occurred and produced a mixture of *N*-terminal-modified derivatives with various chain lengths.[7] *N*-Phenyl-substituted nucleobases, such as 9-phenyladenine, 9-phenylguanine, 1-phenylcytosine, and 1-phenyluracil, are also thought to possess interplanar angles between the phenyl groups and the nucleobases. Thus, we planned to synthesize ONAs consisting of a benzene−phosphate backbone, of which the building blocks are built from nucleobases attached to the benzene residues via the biaryl-like axis.

In this paper, we report the synthesis and properties of novel ONAs consisting of the benzene−phosphate backbone, of which the building blocks $\mathbf{A}^B$, $\mathbf{G}^B$, $\mathbf{C}^B$, and $\mathbf{U}^B$ are built from benzene-core units connected via the biaryl-like axis to the nucleobases (Figure 1).

Results and Discussion

Synthesis of Monomers. To synthesize the ONAs by the phosphoramidite method, appropriately protected phosphoramidites of the analogues $\mathbf{A}^B$, $\mathbf{G}^B$, $\mathbf{C}^B$, and $\mathbf{U}^B$ were synthesized. First, the uridine and cytidine analogues $\mathbf{U}^B$ and $\mathbf{C}^B$ were synthesized according to Scheme 1. 3,5-Bis(hydroxymethyl)aniline (**5**)[8] was treated with TBDMSCl to give the corresponding bis-TBDMS derivative **6** in 74% yield. Compound **6** was reacted with 3-methoxy-2-propenoylisocyanate[9] in DMF and then treated with a solution of 2 M NaOH/EtOH to afford **1** in 45% yield. To synthesize a phosphoramidite, one of the two hydroxyl groups of **1** was protected with a DMTr group to produce a mono-DMTr derivative **8** in 59% yield.

The cytidine analogue **2** was derived from the uridine analogue **1**. After protection of two hydroxyl functions of **1** with a DMTr group, the di-DMTr derivative **10** was treated with 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl) in the presence of DMAP and Et_3N, followed by NH_4OH, to produce a cytosine derivative **11** in 97% yield. Treatment of **11** with 1 M Cl_3CCO_2H/CH_2Cl_2 afforded **2** in 92% yield. To synthesize a phosphoramidite, the hydroxy group of the mono-DMTr derivative **8** was protected with a TBDMS group to give **9** in 95% yield. Compound **9** was converted to a cytosine derivative **12** by the same method described above. After protection of the *exo*-amino function of **12** with a benzoyl (Bz) group, the silyl group was removed by treating with TBAF to afford a mono-DMTr derivative **14** in 97% yield.

The adenosine analogue $\mathbf{A}^B$ was synthesized according to Scheme 2. Treatment of **5** with 5-amino-4,6-dichloropyrimidine in a solution of H_2O/EtOH/concentrated HCl produced **16** in 86% yield. Compound **16** was treated with a mixture of ethyl orthoformate and acetic anhydride, followed by methanolic ammonia in a steel sealed tube at 90°C, to give the adenosine analogue **3** in 36% yield. To synthesize a phosphoramidite, the hydroxyl functions of **16** were protected with a TBDMS group to produce **17** in 70% yield. Compound **17** was treated with a mixture of ethyl orthoformate and acetic anhydride to give **18** in 77% yield. After **18** was treated with methanolic ammonia in a steel sealed tube at 90°C, the *exo*-amino group of **19** was protected with a Bz group to afford **20** in 49% yield. Deprotecting of the silyl groups with TBAF in a solution of THF/CH_2Cl_2 gave a mono-hydroxyl derivative **21** in 53% yield with a recovery of **20** in 44% yield. After the hydroxyl group of **21** was protected with a DMTr group, the TBDMS group was deprotected to afford a mono-DMTr derivative **22** in 84% yield.

The guanosine analogue $\mathbf{G}^B$ was synthesized according to Scheme 3. Treatment of **5** with 2-amino-4-chloro-6-hydroxy-5-phenylazopyrimidine (**23**)[10] in EtOH produced **24** in 70% yield. After cleavage of the diazo linkage of **24** with Zn in an acidic medium, the resulting triaminopyrimidine derivative was treated with *N,N*-dimethylformamide dimethyl acetal and $(EtO)_3CH$ to give the guanosine analogue **4** in 25% yield. To synthesize a phosphoramidite, the hydroxyl functions of **24** were protected with a TBDMS group to produce **25** in 99% yield. Treatment of **25** with Zn in the acidic medium, followed by *N,N*-dimethylformamide dimethyl acetal and $(EtO)_3CH$, afforded a guanine derivative **26** protected with a dimethylaminomethylene (dmf) group in 76% yield. Deprotecting of the silyl group of **26** with TBAF gave a mono-hydroxyl derivative **27** in 59% yield. After the hydroxyl function of **27** was protected with a DMTr group, the TBDMS group was deprotected to afford a mono-DMTr derivative **28** in 86% yield.

The mono-DMTr derivatives **8**, **12**, **22**, and **28** were phosphitylated by the standard procedure[11] to give the corresponding phosphoramidites **29**, **30**, **31**, and **32** in 81, 96, 88, and 60% yields, respectively (Scheme 4). To incorporate the analogues to ends of oligomers, the mono-DMTr derivatives **8**, **12**, **22**, and **28** were modified to the corresponding succinates, which were reacted with controlled pore glasses (CPGs) to produce solid supports **33**−**36** containing **8** (67 μmol/g), **12** (82 μmol/g), **22** (54 μmol/g), and **28** (70 μmol/g), respectively.

Structures of Monomers. To gain insight into the conformational preference of the monomer $\mathbf{U}^B$, X-ray

(8) Behrens, C.; Egholm, M.; Buchardt, O. *Synthesis* **1992**, 1235−1236.
(9) Santana, L.; Teijeira, M.; Uriarte, E. *J. Heterocycl. Chem.* **1999**, *36*, 293−295.
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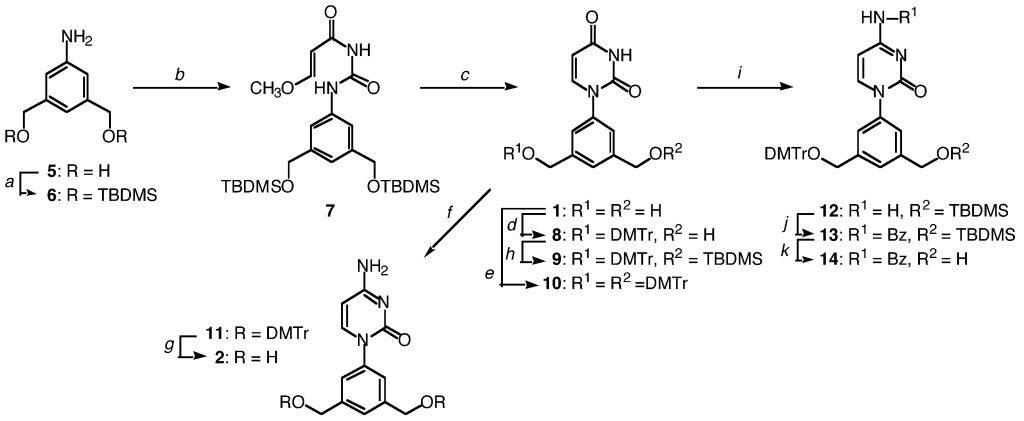
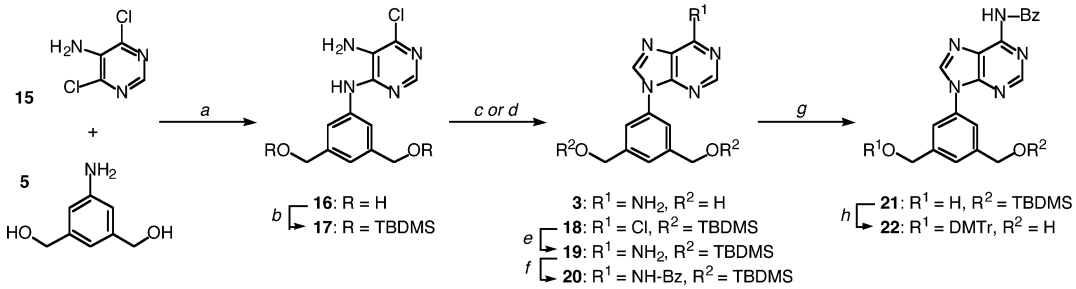
(11) Beaucage, S. L.; Caruthers, M. H. *Tetrahedron Lett.* **1981**, *22*, 1859−1862.

SCHEME 1[a]



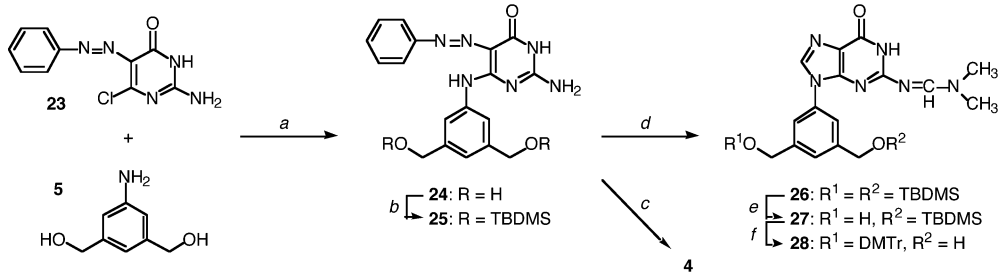
[a] Reagents and conditions: (a) TBDMSCl, imidazole, DMF, rt, 74%; (b) O=C=N−C(=O)-CH=CH−O−CH_3, benzene/DMF, −20 °C to room temperature, 86%; (c) 2 M NaOH/EtOH, 60 °C, 45%; (d) DMTrCl, pyridine, rt, 59%; (e) DMTrCl, DMAP, pyridine, rt, 66%; (f) 2,4,6-triisopropylbenzenesulfonyl chloride, Et_3N, DMAP, CH_3CN, rt, then concd NH_4OH, 0 °C to room temperature, 97%; (g) 1 M CCl_3CO_2H, CH_2Cl_2/THF, rt, 92%; (h) TBDMSCl, imidazole, DMF, rt, 95%; (i) 2,4,6-triisopropylbenzenesulfonyl chloride, Et_3N, DMAP, CH_3CN, rt, then concd NH_4OH, 0 °C to room temperature, 72%; (j) BzCl, pyridine, rt, 92%; (k) TBAF, THF, rt, 97%.

SCHEME 2[a]



[a] Reagents and conditions: (a) concd HCl, EtOH, reflux, 86%; (b) TBDMSCl, imidazole, DMF, rt, 70%; (c) (1) $(EtO)_3CH$, Ac_2O, reflux, (2) NH_3/MeOH, 90 °C, 36%; (d) $(EtO)_3CH$, Ac_2O, reflux, 77%; (e) NH_3/MeOH, 90 °C, 63%; (f) BzCl, pyridine, rt, 49%; (g) TBAF, THF/CH_2Cl_2, rt, 53%; (h) (1) DMTrCl, DMAP, pyridine, rt, (2) TBAF, THF, rt, 84%.

SCHEME 3[a]



[a] Reagents and conditions: (a) EtOH, reflux, 70%; (b) TBDMSCl, imidazole, DMF, rt, 99%; (c) (1) Zn, AcOH/H_2O/EtOH/THF, reflux, (2) $(EtO)_3CH$, 1 M HCl, DMF, rt, 25%; (d) (1) Zn, AcOH/H_2O/EtOH/THF, reflux, (2) *N,N*-dimethylformamide dimethyl acetal, DMF, rt, (3) $(EtO)_3CH$, Ac_2O, rt, 76%; (e) TBAF, THF, rt, 59%; (f) (1) DMTrCl, DMAP, pyridine, rt, (2) TBAF, THF, rt, 86%.

analysis was performed. As shown in Figure 2, the uracil ring forms the plane that is propeller-twisted relative to the benzene plane. It was found that the interplanar angle between the uracil and the benzene moiety is 53.5-(4)°. Structures of the low-energy conformers of the analogues $\mathbf{U}^B$, $\mathbf{C}^B$, $\mathbf{A}^B$, and $\mathbf{G}^B$ were also calculated by MOE using ESFF force field.[12] The interplanar angle between the uracil and the benzene moiety was 56.5°, which was similar to that obtained from X-ray analysis. The interplanar angle between the cytosine and benzene moiety was 54.8°. On the other hand, the interplanar angles between the adenine and the guanine and the benzene moieties were 32.8° and 34.1°, respectively. These values are slightly smaller than those of the pyrimidine analogues.

Structures of base pairs between T and dA, and $\mathbf{U}^B$ and $\mathbf{A}^B$ based on the X-ray analysis and molecular modeling are shown in Figure 3. Although the conformation of the analogues is thought not to be rigid in oligomers, the interplanar angles between the bases and benzene moieties are supposed to influence distances between neighboring base pairs in duplexes.

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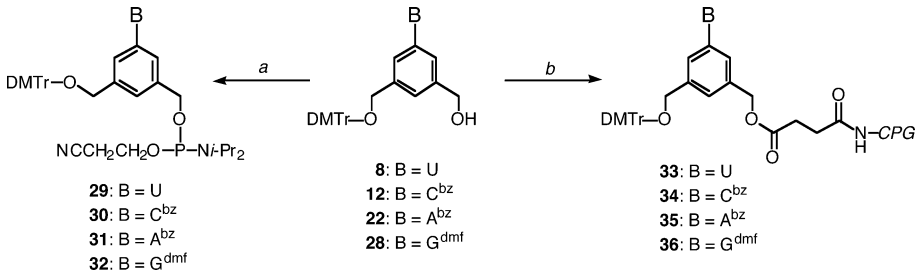
SCHEME 4[a]



[a] Reagents and conditions: (a) Chloro(2-cyanoethoxy)(*N*,*N*-diisopropylamino)phosphine, *N*,*N*-diisopropylethylamine, THF, rt, 81% (**29**), 96% (**30**), 88% (**31**), 60% (**32**); (b) (1) succinic anhydride, DMAP, pyridine, rt, (2) CPG, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, DMF, rt, 67 μmol/g (**33**), 82 μmol/g (**34**), 54 μmol/g (**35**), 70 μmol/g (**36**).

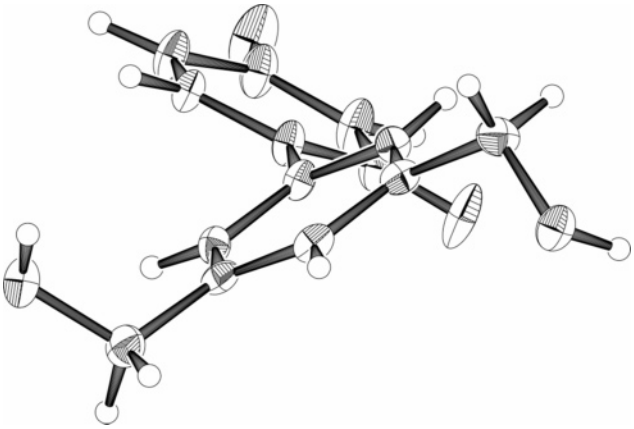


FIGURE 2. ORTEP drawing of $\mathbf{U}^{B}$.

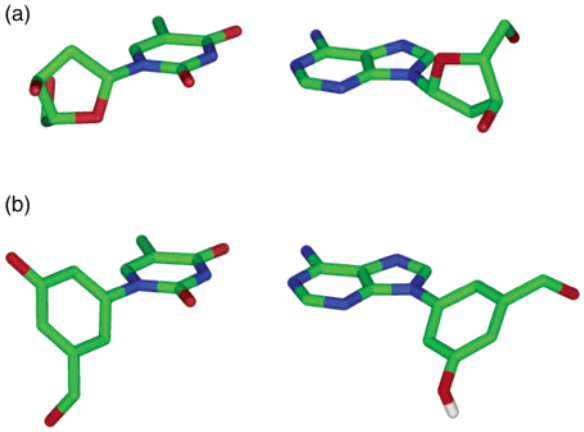


FIGURE 3. Structure of base pairs between T and dA (a) and $\mathbf{U}^{B}$ and $\mathbf{A}^{B}$ (b).

pK_a Measurement of $\mathbf{U}^{B}$. Next, we determined the pK_a of the base moiety of $\mathbf{U}^{B}$ since the pK_a is thought to be an important parameter for understanding the base-pairing properties of the analogues.[13] The pK_a values are listed in Table 1. The pK_a of $\mathbf{U}^{B}$ was 9.3, which was the same as that of rU.

Design of Oligomers. ONAs were synthesized using the phosphoramidite method with a DNA/RNA synthesizer. Sequences of ONAs used in this study are listed in Table 2. The ONAs **7**–**13** are comprised of mixed sequences. The ONAs **9**–**11** contain one base mismatch against the complementary ONA **7**. The ONAs **14** and **15** are composed of homopurine and homopyrimidine sequences, respectively. The ONAs **18**–**21** contain one or three analogues $\mathbf{A}^{B}$ or $\mathbf{U}^{B}$ in the middle of oligodeoxynucleotides (ONs). The ONAs **23** and **24** contain one or three analogues $\mathbf{A}^{B}$ in the middle of oligonucleotides (2′-*O*-Me-ONs) composed of 2′-*O*-methylribonucleosides. The fully protected ONAs were treated with concentrated NH_4OH at 55°C, and the released ONAs were purified by denaturing 20% polyacrylamide gel electrophoresis to afford deprotected ONAs. These ONAs were analyzed by MALDI-TOF/MS and the observed molecular weights supported their structures.

TABLE 1. pK_a Values of Uridine and its Analogue[a]

compound	pK_a
rU	9.3 (9.2)[b]
U^{B}	9.3

[a] A 0.2 M NaCl solution was used for all pH ranges instead of buffer. [b] Data taken from Dawson et al.[14]

UV Melting Studies of Duplexes. The thermal stabilities of the duplexes composed of the benzene–phosphate backbone were compared with those of the natural ONs. Thermal denaturation was performed in a buffer of 10 mM sodium phosphate (pH 7.0) containing 1.0 M NaCl.

The thermal stabilities of the duplexes were highly dependent on their sequences. The UV melting profiles of the duplexes are shown in Figure 4. The melting temperatures (T_ms) of the duplexes **4** and **5** comprised of the benzene–phosphate backbone were 38.4 and 48.8 °C (Figure 4a and b), whereas those of the duplexes **1** and **2** consisting of the natural nucleosides were 49.1 and 51.7 °C, respectively. Thus, it was found that the duplexes with the benzene–phosphate backbone composed of the mixed sequences are thermally less stable than the natural DNA duplexes. However, as shown in Figure 4d, the T_m value of the duplex **4** was apparently higher than those of the duplexes between the ONA **7** and ONA **9**, **10**, or **11** containing one mismatch base pair. The result implies that the ONAs composed of the benzene–phosphate backbone retain enough base selectivity.

On the other hand, the T_m value of the duplex **6** between $(\mathbf{U}^{B})_{12}$ and $(\mathbf{A}^{B})_{12}$ was 48.3 °C, whereas that of a natural T_{12}:dA_{12} duplex was 41.8 °C (Figure 4c). Thus, it was revealed that the duplex between $(\mathbf{U}^{B})_{12}$ and $(\mathbf{A}^{B})_{12}$ composed of the homopyrimidine and homopurine se-

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TABLE 2. Sequences of Oligomers[a]

No. of Duplex	No. of ON and ONA	sequence
Duplex**1**	ON**1**	5′-d(GTCAATAATCTG)-3′
	ON**2**	3′-d(CAGTTATTAGAC)-5′
Duplex**2**	ON**3**	5′-d(AACGGATTACAA)-3′
	ON**4**	3′-d(TTGCCTAATGTT)-5′
Duplex**3**	ON**5**	5′-d(AAAAAAAAAAAA)-3′
	ON**6**	3′-d(TTTTTTTTTTTT)-5′
Duplex**4**	ONA**7**	b(GUCAAUAAUCUG)
	ONA**8**	b(CAGUUAUUAGAC)
	ONA**9**	b(CAGUUC̲UUAGAC)
	ONA**10**	b(CAGUUG̲UUAGAC)
	ONA**11**	b(CAGUUAC̲UAGAC)
Duplex**5**	ONA**12**	b(AACGGAUUACAA)
	ONA**13**	b(UUGCCUAAUGUU)
Duplex**6**	ONA**14**	b(AAAAAAAAAAAA)
	ONA**15**	b(UUUUUUUUUUUU)
Duplex**7**	ON**16**	5′-d(GCACCGAAAAAACCACG)-3′
	ON**17**	3′-d(CGTGGCTTTTTTGGTGC)-5′
Duplex**8**	ONA**18**	5′-d(GCACCGAAAA^{B}AACCACG)-3′
	ONA**19**	3′-d(CGTGGCTTTU^{B}TTGGTGC)-5′
Duplex**9**	ONA**20**	5′-d(GCACCGAAA^{B}A^{B}A^{B}ACCACG)-3′
	ONA**21**	3′-d(CGTGGCTTU^{B}U^{B}U^{B}TGGTGC)-5′
Duplex**10**	2′-*O*-Me-ON**22**	5′-[2′-*O*-Me(GCACCGAAAAAACCACG)]-3′
	ON**17**	3′-d(CGTGGCTTTTTTGGTGC)-5′
Duplex**11**	2′-*O*-Me-ONA**23**	5′-[2′-*O*-Me(GCACCGAAAA^{B}AACCACG)]-3′
	ONA**19**	3′-d(CGTGGCTTTU^{B}TTGGTGC)-5′
Duplex**12**	2′-*O*-Me-ONA**24**	5′-[2′-*O*-Me(GCACCGAAA^{B}A^{B}A^{B}ACCACG)]-3′
	ONA**21**	3′-d(CGTGGCTTU^{B}U^{B}U^{B}TGGTGC)-5′

[a] b indicates the oligomers composed of the benzene−phosphate backbone; underlined letters show the mismatch bases against ONA**7**.

quences was thermally more stable than the corresponding natural DNA duplex. Thermodynamic parameters of the duplexes **3** and **6** on duplex formations were determined by calculations based on the slope of a $1/T_m$ versus $\ln(C_T/4)$ plot, where C_T is the total concentration of the single strands.[15] The parameters are summarized in Table 3. The $\Delta G°_{37}$ values of the duplexes **3** and **6** were −9.1 and −10.8 kcal/mol, respectively. Both the $\Delta H°$ and $\Delta S°$ values of the duplex **6** were smaller than those of the duplex **3**. This implies that the duplex formation between the ONs **14** and **15** is less favorable in entropy but more favorable in enthalpy than that between the ONs **5** and **6** consisting of the natural nucleosides. The disadvantage in entropy in the duplex formation between the ONAs **14** and **15** is compensated by the enthalpy term. The $\Delta\Delta H°$ value between the duplexes **6** and **3** was 15.6 kcal/mol. These results suggest that the duplex **6** between the ONAs **14** and **15** with the benzene−phosphate backbone is thermally and thermodynamically stabilized by the stacking interaction of the benzene moiety of each unit with the neighboring benzene or base moieties.

Global Conformations of Duplexes. To investigate the influence of the $\mathbf{A}^{B}$:$\mathbf{U}^{B}$ base pair on the duplex formation in detail, we next prepared the oligodeoxynucleotides, ONAs **18**−**21**, and the oligonucleotides comprised of 2′-*O*-methylribonucleotides, 2′-*O*-Me-ONAs **23** and **24**, which contain the analogue $\mathbf{A}^{B}$ or $\mathbf{U}^{B}$ in the middle of their strands (Table 2). To study the global conformations of the duplexes **8**, **9**, **11**, and **12** between ONA and ONA, and 2′-*O*-Me-ONA and ONA, CD spectra of the duplexes were measured. Generally, a B-form duplex shows a positive CD band around 280 nm and a negative band around 240 nm, whereas an A-form duplex reveals a positive peak around 270 nm and a negative peak at 210 nm.

The spectrum of the duplex **7** between the ONs **16** and **17** showed the positive CD band at 278 nm and the negative band at 248 nm, which were attributable to the B-form duplex (Figure 5). Although the intensity of the negative CD band of the duplex **8** containing one $\mathbf{A}^{B}$:$\mathbf{U}^{B}$

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(15) Marky, L. A.; Breslauer, K. L. *Biopolymer* **1987**, *26*, 1601−1620.

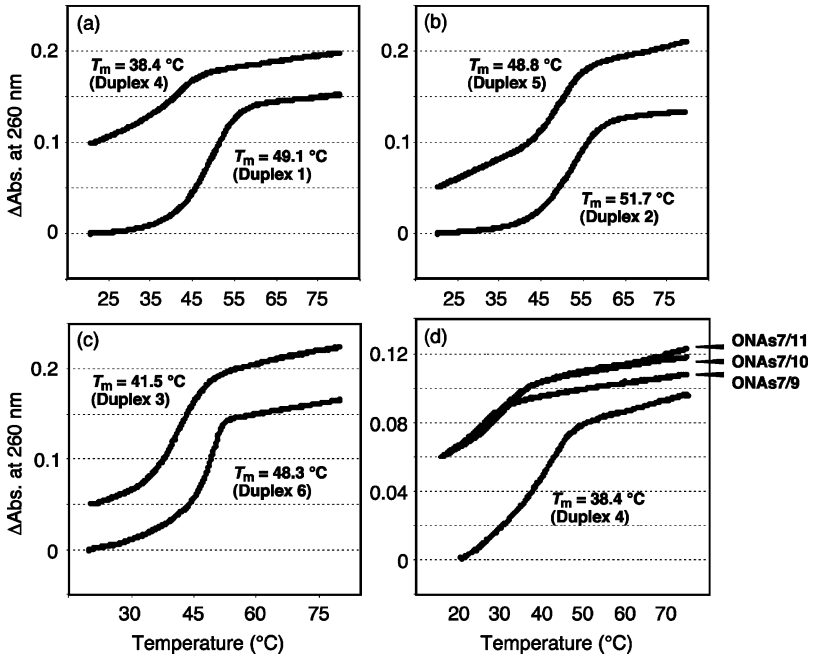

FIGURE 4. UV melting profiles of duplexes and their T_ms. Thermal denaturation was performed in a buffer comprised of 10 mM sodium phosphate (pH 7.0) and 1.0 M NaCl.

TABLE 3. Thermodynamic Parameters[a]

	T_m[b] (°C)	ΔT_m (°C)	$-\Delta H°$ (kcal/mol)	$-\Delta S°$ (cal/K·mol)	$-\Delta G°_{37}$ (kcal/mol)
duplex **3**	41.5		56.4 ± 1.1	152.5 ± 2.9	9.1
duplex **6**	48.3	6.8	72.0 ± 2.0	197.3 ± 5.4	10.8
duplex **7**	63.6		114.2 ± 5.1	312.6 ± 13.9	17.3
duplex **8**	53.2	−10.4	126.6 ± 5.3	361.2 ± 15.0	14.6
duplex **9**	51.3	−12.3	162.9 ± 9.0	475.6 ± 26.2	15.5
duplex **10**	59.3		78.8 ± 1.3	210.5 ± 3.6	13.5
duplex **11**	52.8	−6.5	86.4 ± 1.6	238.5 ± 4.3	12.5
duplex **12**	49.2	−10.1	87.1 ± 1.3	243.8 ± 3.5	11.5

[a] Thermal denaturation was performed in a buffer (10 mM sodium phosphate, pH 7.0) containing 1.0 M NaCl for duplexes **3** and **6**, or containing 0.1 M NaCl for duplexes **7**−**12**. The standard deviations for $\Delta H°$ and $\Delta S°$ were estimated from the linearity of the T_m^{-1} versus $\ln(C_T/4)$ plots. [b] T_m values at 3 μM duplex concentrations.

base pair was slightly less than that of the duplex **7**, the duplex was found to retain the B-like conformation. When three $\mathbf{A}^B$:$\mathbf{U}^B$ base pairs were introduced into the duplex, intensities of both the positive and negative CD bands decreased as compared to those of the duplex **7**. In addition, the negative CD band shifted to around 254 nm, and a new CD band appeared around 240 nm. This indicates that the global conformation of the DNA B-form duplex is disordered by introducing three $\mathbf{A}^B$:$\mathbf{U}^B$ base pairs.

On the other hand, the spectrum of the duplex **10** revealed the positive CD band at 267 nm and the negative band at 209 nm, which were attributable to the A-form duplex. Although the intensities of the positive CD bands of the duplexes **11** and **12** containing the $\mathbf{A}^B$:$\mathbf{U}^B$ base pairs were slightly less than that of the duplex **10**, those duplexes were found to retain the A-like conformation.

Thermodynamic Parameters of Duplexes. Thermodynamic parameters of the duplexes **7**−**12** on duplex formations were determined by the same method described above. The parameters are summarized in Table 3. In the case of the ONA/ONA duplex, the T_m and $-\Delta G°_{37}$ values of the duplexes **8** and **9** were smaller than those of the duplex **7**. Thus, it was found that the ONA/ONA duplexes are thermally and thermodynamically destabilized by introducing the $\mathbf{A}^B$:$\mathbf{U}^B$ base pair. However, the $-\Delta G°_{37}$ value of the duplex **9** was greater than that of the duplex **8**. This implies that the duplex **9** containing three $\mathbf{A}^B$:$\mathbf{U}^B$ base pairs is thermodynamically more stable than the duplex **8** containing one $\mathbf{A}^B$:$\mathbf{U}^B$ base pair. The $-\Delta H°$ values became greater as the numbers of the $\mathbf{A}^B$:$\mathbf{U}^B$ base pairs increased, although the $-\Delta S°$ values also became larger as the numbers of the $\mathbf{A}^B$:$\mathbf{U}^B$ base pairs increased. The $\Delta\Delta H°$ value between the duplexes **9** and **7** was 48.7 kcal/mol. The result indicates that the benzene moieties of the analogues interact with the neighboring benzene or base moieties by the stacking interaction in the ONA/ONA duplex.

In the case of the 2′-*O*-Me-ONA/ONA duplex, the T_m and $-\Delta G°_{37}$ values became smaller as the numbers of the $\mathbf{A}^B$:$\mathbf{U}^B$ base pairs increased. Thus, it was found that the 2′-*O*-Me-ONA/ONA duplexes are also thermally and thermodynamically destabilized by introducing the $\mathbf{A}^B$:$\mathbf{U}^B$ base pair. It was revealed that the $\Delta\Delta H°$ value (8.3 kcal/mol) between the duplexes **10** and **12** is not so large as compared to that between the duplexes **7** and **9**, although the $\Delta\Delta S°$ value (33.3 cal/K·mol) between the duplexes **10** and **12** is smaller than that (163.0 cal/K·mol) between the duplexes **7** and **9**. The result suggests that the stacking interaction of the benzene moiety of the analogues in the 2′-*O*-Me-ONA/ONA duplex is weaker than that in the ONA/ONA duplex.

In conclusion, we have demonstrated the synthesis of nucleic acid analogues consisting of a benzene−phosphate backbone. It was found that the thermal stabilities of the duplexes composed of the benzene−phosphate backbone are highly dependent on their sequences. The duplexes

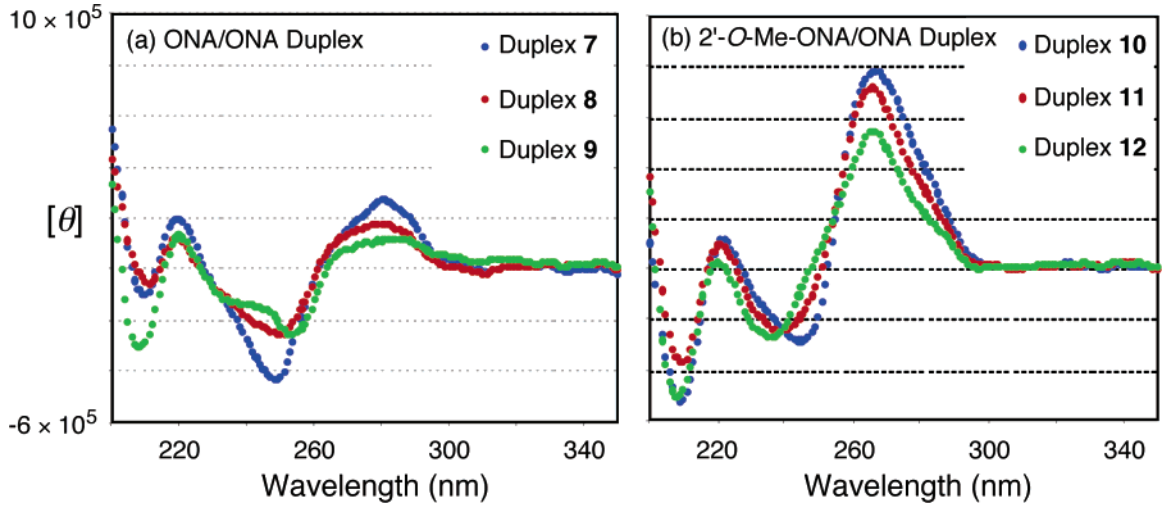

FIGURE 5. CD spectra of duplexes. (a) ONA/ONA duplex; (b) 2′-*O*-Me-ONA/ONA duplex.

with the benzene−phosphate backbone comprised of the mixed sequences were thermally less stable than the natural DNA duplexes, whereas the duplex between $(\mathbf{U}^{B})_{12}$ and $(\mathbf{A}^{B})_{12}$ composed of the homopyrimidine and homopurine sequences was thermally and thermodynamically more stable than the corresponding natural DNA duplex. Additionally, it was suggested that the benzene moieties of the analogues more efficiently interact with the neighboring benzene or base moieties in the ONA/ONA duplex than in the 2′-*O*-Me-ONA/ONA duplex. Thus, the duplexes consisting of the benzene−phosphate backbone, especially those composed of the homopyrimidine and homopurine sequences, may offer a novel structural motif useful for developing novel materials and probes, such as DNA-based nanowires[16] and molecular beacons,[17] applicable to the fields of bio- and nanotechnologies.

Materials and Methods

General Remarks. NMR spectra were recorded at 400 MHz (^{1}H), at 100 MHz (^{13}C), and at 162 MHz (^{31}P) and are reported in ppm downfield from TMS or 85% H_3PO_4. *J* values are given in hertz. Mass spectra were obtained by electron ionization (EI) or fast atom bombardment (FAB) method.

3,5-Bis(*tert*-butyldimethylsilanyloxymethyl)aniline (6). A mixture of 3,5-bis(hydroxymethyl)aniline (**5**)[8] (1.35 g, 8.82 mmol), TBDMSCl (2.92 g, 19.4 mmol), and imidazole (2.64 g, 38.8 mmol) in DMF (18 mL) was stirred at room temperature. After 22 h, EtOH (2 mL) was added to the mixture, and the whole was stirred for 10 min. The mixture was partitioned between EtOAc and H_2O. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (SiO_2, 10−50% EtOAc in hexane) to give **6** (2.50 g, 74% as a yellow oil): ^{1}H NMR ($CDCl_3$) δ 6.66 (s, 1H), 6.55 (s, 2H), 4.65 (s, 4H), 3.64 (s, 2H), 0.95 (s, 18H), 0.09 (s, 12H); ^{13}C NMR (DMSO-d_6) δ 148.3, 141.5, 111.7, 110.4, 64.7, 25.8, 18.0, −5.3; LRMS (EI) *m/z* 381 (M^+); HRMS (EI) calcd for $C_{20}H_{39}NO_2Si_2$ 381.2519, found 381.2516.

***N*-[3,5-Bis(*tert*-butyldimethylsilanyloxymethyl)phenylcarbamoyl]-3-methoxy-2-propenamide (7).** A solution of 3-methoxy-2-propenoylisocyanate[9] in benzene (78 mL, 31.3 mmol) was added to a solution of **6** (3.70 g, 9.71 mmol) in DMF (38 mL) at −20 °C with 4 Å molecular sieves. After the addition, the mixture was allowed to warm to room temperature while stirring overnight. The molecular sieves were filtered off, the solvent was evaporated in vacuo, and the resulting residue was purified by column chromatography (SiO_2, 10−25% EtOAc in hexane) to give **7** (4.22 g, 86% as a white solid): mp 150 °C; ^{1}H NMR ($CDCl_3$) δ 10.78 (s, 1H), 9.07 (s, 1H), 7.76 (d, 1H, *J* = 12.4), 7.36−7.11(s, 3H), 5.36 (d, 1H, *J* = 12.4), 4.71 (s, 4H), 3.75 (s, 3H), 0.94 (s, 18H), 0.10 (s, 12H); ^{13}C NMR (DMSO-d_6) δ 168.2, 163.6, 151.2, 142.1, 137.5, 118.5, 115.4, 97.7, 64.1, 58.2, 25.8, 18.0, −5.3; LRMS (FAB) *m/z* 509 (MH^+); HRMS (FAB) calcd for $C_{25}H_{45}N_2O_5Si_2$ 509.2789, found 509.2880. Anal. Calcd for $C_{25}H_{44}N_2O_5Si_2$: C, 59.02; H, 8.72; N, 5.51. Found: C, 58.81; H, 8.87; N, 5.51.

1-[3,5-Bis(hydroxymethyl)phenyl]uracil (1). A mixture of **7** (0.322 g, 0.63 mmol), 2 M NaOH (2.6 mL), and EtOH (2.6 mL) was stirred at 60 °C for 1 h. After the solution was cooled, it was neutralized with AcOH. The solvent was evaporated in vacuo, and the resulting residue was purified by column chromatography (SiO_2, 6−9% MeOH in $CHCl_3$) to give **1** (71 mg, 45% as a white solid): mp 201−203 °C; UV λmax (H_2O) 220 nm, 273 nm; ^{1}H NMR (DMSO-d_6) δ 11.42 (s, 1H), 7.65 (d, 1H, *J* = 8.0), 7.32−7.17 (s, 3H), 5.65 (d, 1H, *J* = 8.0), 5.31 (t, 2H, *J* = 5.6), 4.52 (d, 4H, *J* = 5.6); ^{13}C NMR (DMSO-d_6) δ 163.7, 150.4, 145.5, 143.7, 138.7, 124.0, 122.8, 101.6, 62.7; LRMS (EI) *m/z* 248 (M^+); HRMS (EI) calcd for $C_{12}H_{12}N_2O_4$ 248.0797, found 248.0790. Anal. Calcd for $C_{12}H_{12}N_2O_4$: C, 58.06; H, 4.87; N, 11.29. Found: C, 58.00; H, 4.87; N, 11.24.

1-[3-(4,4′-Dimethoxytrityloxymethyl)-5-(hydroxymethyl)phenyl]uracil (8). A mixture of **1** (0.23 g, 0.93 mmol) and DMTrCl (0.38 g, 1.12 mmol) in pyridine (5 mL) was stirred at room temperature for 4 h. The mixture was partitioned between $CHCl_3$ and H_2O. The organic layer was washed with aqueous $NaHCO_3$ (saturated) and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (SiO_2, 0−9% MeOH in $CHCl_3$) to give **8** (0.302 g, 59%): mp 85−87 °C; ^{1}H NMR (DMSO-d_6) δ 11.41 (s, 1H), 7.67 (d, 1H, *J* = 8.0), 7.44−6.89 (m, 16H), 5.64 (d, 1H, *J* = 8.0), 5.32 (t, 1H, *J* = 5.4), 4.53 (d, 2H, *J* = 5.4), 4.09 (s, 2H), 3.72 (s, 6H); ^{13}C NMR (DMSO-d_6) δ 163.7, 158.1, 150.4, 145.5, 144.8, 144.0, 139.6, 138.8, 135.5, 129.7, 128.0, 127.6, 126.8, 124.3, 123.4, 123.3, 113.4, 101.6, 86.0, 79.2, 64.6, 62.3, 55.0; LRMS (FAB) *m/z* 551 (MH^+); HRMS(FAB) calcd for $C_{33}H_{31}N_2O_6$ 551.2104, found 551.2176. Anal. Calcd for $C_{33}H_{30}N_2O_6 \cdot 1/2H_2O$: C, 70.83; H, 5.58; N, 5.01. Found: C, 70.62; H, 5.78; N, 4.74.

1-[3-(*tert*-Butyldimethylsilanyloxymethyl)-5-(4,4′-dimethoxytrityloxymethyl)phenyl]uracil (9). A mixture of **8** (0.44 g, 0.79 mmol), TBDMSCl (0.13 g, 0.87 mmol), and imidazole

(16) Tanaka, K.; Tengeiji, A.; Kato, T.; Toyama, N.; Shionoya, M. *Science* **2003**, *299*, 1212−1213.
(17) Tyagi, S.; Kramer, F. R. *Nat. Biotechnol.* **1996**, *14*, 303−308.

(0.12 g, 1.74 mmol) in DMF (1.6 mL) was stirred at room temperature for 1 h. The mixture was partitioned between $CHCl_3$ and H_2O. The organic layer was washed with aqueous $NaHCO_3$ (saturated) and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (SiO_2, 10% EtOAc in hexane) to give **9** (0.501 g, 95%): mp 62−64 °C; ^{1}H NMR (DMSO-d_6) δ 11.42 (s, 1H), 7.67 (d, 1H, J = 8.0), 7.49−6.89 (m, 16H), 5.65 (q, 1H, J = 2.0 and 8.0), 4.76 (s, 2H), 4.10 (s, 2H), 3.73 (s, 6H), 0.91 (s, 9H), 0.11 (s, 6H); LRMS (FAB) *m/z* 665 (MH^+); HRMS (FAB) calcd for $C_{39}H_{45}N_2O_6Si$, 665.3047 found 665.3043. Anal. Calcd for $C_{39}H_{44}N_2O_6Si\cdot 3/4H_2O$: C, 69.05; H, 6.76; N, 4.13. Found: C, 69.14; H, 6.83; N, 3.76.

1-[3-(*tert*-Butyldimethylsilanyloxymethyl)-5-(4,4′-dimethoxytrityloxymethyl)phenyl]cytosine (12). A mixture of **9** (0.45 g, 0.68 mmol), Et_3N (0.19 mL, 1.35 mmol), DMAP (0.17 g, 1.35 mmol), and 2,4,6-triisopropylbenzenesulfonyl chloride (0.41 g, 1.35 mmol) in CH_3CN (3.4 mL) was stirred at room temperature for 1 h. The mixture was cooled in an ice-bath. Concentrated NH_4OH (25%, 1.6 mL) was added, and the mixture was stirred at room temperature for 19 h. The mixture was evaporated, and the residue was partitioned between $CHCl_3$ and H_2O. The organic layer was washed with aqueous $NaHCO_3$ (saturated) and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (SiO_2, 2−4% MeOH in $CHCl_3$) to give **12** (0.32 g, 72%): ^{1}H NMR (DMSO-d_6) δ 7.58 (d, 1H, J = 7.0), 7.44−6.88 (m, 16H), 5.74 (d, 1H, J = 7.0), 4.75 (s, 2H), 4.08 (s, 2H), 3.72 (s, 6H), 0.90 (s, 9H), 0.10 (s, 6H); ^{13}C NMR (DMSO-d_6) δ 166.1, 158.1, 154.8, 145.7, 144.9, 142.2, 141.1, 139.5, 135.5, 129.6, 127.9, 127.6, 126.8, 123.2, 122.9, 122.8, 113.3, 94.1, 86.0, 64.5, 63.8, 55.0, 25.8, 18.0, −5.3; LRMS (FAB) *m/z* 664 (MH^+); HRMS (FAB) calcd for $C_{39}H_{46}N_3O_5Si$ 664.3207, found 664.3211. Anal. Calcd for $C_{39}H_{45}N_3O_5Si$: C, 70.56; H, 6.83; N, 6.33. Found: C, 70.38; H, 6.81; N, 6.13.

4-*N*-Benzoyl-1-[3-(*tert*-Butyldimethylsilanyloxymethyl)-5-(4,4′-dimethoxytrityloxymethyl)phenyl]cytosine (13). A mixture of **12** (0.25 g, 0.38 mmol) and BzCl (0.13 mL, 0.77 mmol) in pyridine (2 mL) was stirred at room temperature for 1 h. The mixture was partitioned between $CHCl_3$ and aqueous $NaHCO_3$ (saturated). The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (SiO_2, 25−100% EtOAc in hexane) to give **13** (0.27 g, 92%): ^{1}H NMR (DMSO-d_6) δ 11.34 (s, 1H), 8.15 (d, 1H, J = 6.4), 8.02 (d, 2H, J = 7.2), 7.65−6.89 (m, 20H), 4.79 (s, 2H), 4.13 (s, 2H), 3.73 (s, 6H), 0.92 (s, 9H), 0.12 (s, 6H); ^{13}C NMR (DMSO-d_6) δ 158.1, 144.9, 142.6, 140.0, 139.8, 135.5, 132.8, 129.7, 128.5, 128.4, 127.9, 127.6, 126.8, 123.7, 123.2, 122.7, 113.3, 86.0, 64.5, 63.7, 59.7, 55.0, 25.8, 20.7, 18.0, 14.1, −5.3; LRMS (FAB) *m/z* 768 (MH^+); HRMS (FAB) calcd for $C_{46}H_{50}N_3O_6Si$ 768.3469, found 768.3476. Anal. Calcd for $C_{46}H_{49}N_3O_6Si\cdot 2/3H_2O$: C, 70.83; H, 6.50; N, 5.39. Found: C, 70.68; H, 6.51; N, 5.22.

4-*N*-Benzoyl-1-[3-(4,4′-dimethoxytrityloxymethyl)-5-(hydroxymethyl)phenyl]cytosine (14). A mixture of **13** (0.31 g, 0.41 mmol) and TBAF (1 M in THF, 0.82 mL, 0.82 mmol) in THF (2.4 mL) was stirred at room temperature for 19 h. The solvent was evaporated, and the resulting residue was purified by column chromatography (SiO_2, 2% MeOH in $CHCl_3$) to give **14** (0.26 g, 97%): ^{1}H NMR (DMSO-d_6) δ 11.35 (s, 1H), 8.17 (d, 1H, J = 7.2), 8.02 (d, 2H, J = 7.6), 7.65−6.90 (m, 20H), 5.37 (t, 1H, J = 6.0), 4.56 (d, 2H, J = 6.0), 4.12 (s, 2H), 3.73 (s, 6H); ^{13}C NMR (DMSO-d_6) δ 158.1, 144.8, 143.9, 139.6, 135.5, 132.8, 129.7, 128.5, 128.0, 127.6, 126.8, 124.4, 123.1, 122.9, 113.3, 86.1, 79.2, 64.6, 62.3, 55.0; LRMS (FAB) *m/z* 654 (MH^+); HRMS (FAB) calcd for $C_{40}H_{36}N_3O_6$ 654.2604, found 654.2610. Anal. Calcd for $C_{40}H_{35}N_3O_6\cdot 5/4H_2O$: C, 70.81; H, 5.61; N, 6.19. Found: C, 71.19; H, 5.67; N, 5.77.

1-[3,5-Bis(4,4′-dimethoxytrityloxymethyl)phenyl]-uracil (10). A mixture of **1** (0.62 g, 2.5 mmol), DMTrCl (1.69 g, 5.0 mmol), and DMAP (15 mg, 0.125 mmol) in pyridine (13 mL) was stirred at room temperature for 18 h. The mixture was partitioned between $CHCl_3$ and aqueous $NaHCO_3$ (saturated). The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (SiO_2, 25−100% EtOAc in hexane) to give **10** (1.4 g, 66%):^{1}H NMR (DMSO-d_6) δ 11.42 (s, 1H), 7.66 (d, 1H, J = 7.6), 7.51−6.86 (m, 29H), 5.64 (d, 1H, J = 7.6), 4.14 (s, 4H), 3.71 (s, 12H); ^{13}C NMR (DMSO-d_6) δ 163.1, 158.5, 150.0, 144.8, 144.7, 141.4, 138.1, 136.0, 130.0, 128.1, 127.9, 126.9, 125.7, 123.1, 113.2, 102.4, 86.7, 64.9, 55.2; LRMS (FAB) *m/z* 853 (MH^+); HRMS (FAB) calcd for $C_{54}H_{49}N_2O_8$ 853.3489, found 853.3505.

1-[3,5-Bis(4,4′-dimethoxytrityloxymethyl)phenyl]-cytosine (11). Compound **10** (0.20 g, 0.23 mmol) was treated as described in the preparation of **12** to give **11** (0.19 g, 97%): ^{1}H NMR (DMSO-d_6) δ 7.77 (d, 1H, J = 7.4), 7.60−6.86 (m, 31H), 5.75 (d, 1H, J = 7.4), 4.13 (s, 4H), 3.71 (s, 12H); ^{13}C NMR (DMSO-d_6) δ 166.0, 158.5, 156.3, 145.8, 144.9, 140.7, 140.6, 136.1, 130.0, 128.1, 127.9, 126.8, 124.9, 123.4, 113.2, 94.4, 86.6, 65.1, 55.2; LRMS (FAB) *m/z* 852 (MH^+); HRMS (FAB) calcd for $C_{54}H_{50}N_3O_7$ 852.3649, found 852.3642.

1-[3,5-Bis(hydroxymethyl)phenyl]cytosine (2). A 1 M solution ofCCl_3CO_2H in CH_2Cl_2 (10 mL) was added to a solution of **11** (0.15 g, 0.176 mmol) in THF (1.8 mL). The mixture was stirred at room temperature for 18 h. The mixture was partitioned between $CHCl_3$ and H_2O. The water layer was concentrated, and the product was washed with H_2O to give **2** (40 mg, 92%): mp 132−135 °C; UV λmax (H_2O) 202 nm, 246 nm, 286 nm; ^{1}H NMR (DMSO-d_6) δ 7.58 (d, 1H, J = 7.2), 7.25 (s, 1H), 7.09 (s, 1H), 5.79 (s, 1H), 5.27 (t, 2H, J = 5.8), 4.50 (d, 4H, J = 5.8); ^{13}C NMR (DMSO-d_6) δ 166.1, 154.9, 145.8, 143.3, 141.1, 123.1, 122.6, 93.9, 62.5; LRMS (EI) *m/z* 247 (M^+); HRMS (EI) calcd for $C_{12}H_{13}N_3O_3$ 247.0957, found 247.0967. Anal. Calcd for $C_{12}H_{13}N_3O_3$: C, 58.29; H, 5.30; N, 17.00. Found: C, 58.11; H; 5.49, N, 16.89.

5-Amino-4-[3,5-bis(hydroxymethyl)anilino]-6-chloropyrimidine (16). A mixture of **5** (0.728 g, 4.76 mmol), 5-amino-4,6-dichloropyrimidine (**15**) (0.702 g, 4.28 mmol), concentrated HCl (0.18 mL), and EtOH (1.8 mL) was refluxed for 7 h. H_2O (10 mL) was added to the solution, and the mixture was allowed to stand overnight in the refrigerator. The product was filtered and washed with H_2O to give **16** (1.03 g, 86%): mp 243−245 °C; ^{1}H NMR (DMSO-d_6) δ 8.57 (s, 1H), 7.86 (s, 1H), 7.56 (s, 2H), 6.93 (s, 1H), 5.44 (s, 2H), 5.18 (s, 2H), 4.47 (s, 4H); ^{13}C NMR (DMSO-d_6) δ 148.8, 144.7, 143.7, 142.7, 139.5, 138.3, 124.7, 118.9, 116.8, 63.0; LRMS (EI) *m/z* 280 (M^+); HRMS (EI) calcd for $C_{12}H_{13}N_4ClO_2$ 280.0727, found 280.0723. Anal. Calcd for $C_{12}H_{13}N_4ClO_2$: C, 51.34; H, 4.67; N, 19.96. Found: C, 51.09; H, 4.67; N, 19.87.

5-Amino-4-[3,5-bis(*tert*-butyldimethylsilanyloxymethyl)-anilino]-6-chloropyrimidine (17). A mixture of **16** (1.08 g, 3.83 mmol), TBDMSCl (1.27 g, 8.45 mmol), and imidazole (1.14 g, 16.7 mmol) in DMF (8 mL) was stirred at room temperature for 24 h. The mixture was partitioned between EtOAc and H_2O. The organic layer was washed with aqueous $NaHCO_3$ (saturated) and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (SiO_2, 20−50% EtOAc in hexane) to give **17** (1.33 g, 70%): mp 167−169 °C; ^{1}H NMR (DMSO-d_6) δ 8.57 (s, 1H), 7.70 (s, 2H), 6.94 (s, 1H), 5.45 (s, 2H), 4.68 (s, 4H), 0.90 (s, 18H), 0.08 (s, 12H); ^{13}C NMR (DMSO-d_6) δ 149.2, 145.0, 142.0, 140.1, 139.0, 125.3, 118.5, 117.2, 64.9, 26.3, 18.5, 4.8; LRMS (EI) *m/z* 508 (M^+); HRMS (EI) calcd for $C_{24}H_{41}N_4ClO_2Si_2$ 508.2457, found 508.2463. Anal. Calcd for $C_{24}H_{41}N_4ClO_2Si_2$: C, 56.61; H, 8.12; N, 11.00. Found: C, 56.48; H, 8.07; N, 10.95.

9-[3,5-Bis(*tert*-butyldimethylsilanyloxymethyl)phenyl]-6-chloropurine (18). A mixture of **17** (0.303 g, 0.61 mmol), $(EtO)_3CH$ (1.4 mL), and Ac_2O (1.4 mL) was refluxed for 6 h. The solvent was evaporated in vacuo, and the resulting residue was purified by column chromatography (SiO_2, 17% EtOAc in hexane) to give **18** (0.242 g, 77%): mp 91−93 °C; ^{1}H NMR ($CDCl_3$) δ 8.80 (s, 1H), 8.41 (s, 1H), 7.58 (s, 2H), 7.34 (s, 1H), 4.84 (s, 4H), 0.96 (s, 18H), 0.13 (s, 12H); ^{13}C NMR ($CDCl_3$) δ

152.6, 151.6, 151.5, 144.2, 144.0, 134.0, 132.2, 123.4, 119.2, 64.2, 25.9, 18.4, −5.3; LRMS (FAB) *m/z* 519 (MH^+); HRMS (FAB) calcd for $C_{25}H_{40}N_4ClO_2Si_2$ 519.2378, found 519.2388. Anal. Calcd for $C_{25}H_{39}N_4ClO_2Si_2$: C, 57.83; H, 7.57; N, 10.79. Found: C, 57.85; H, 7.50; N, 10.68.

9-[3,5-Bis(*tert*-butyldimethylsilanyloxymethyl)phenyl]-adenine (19). A solution of **18** (101 mg, 0.19 mmol) in methanolic ammonia (10 mL, saturated at 0 °C) in a steel sealed tube was heated to 90 °C for 6 h. After the tube was cooled to room temperature then degassed, the solvent was evaporated in vacuo. The resulting residue was purified by column chromatography (SiO_2, 20−100% EtOAc in hexane) to give **19** (59 mg, 63%): mp 122−124 °C; ^{1}H NMR ($CDCl_3$) δ 8.40 (s, 1H), 8.08 (s, 1H), 7.54 (s, 2H), 7.36 (s, 1H), 5.85 (s, 2H), 4.83 (s, 4H), 0.96 (s, 18H), 0.13 (s, 12H); ^{13}C NMR ($CDCl_3$) δ 155.6, 153.6, 150.0, 143.6, 139.7, 134.7, 122.9, 120.1, 119.3, 64.4, 25.9, 18.4, −5.3; LRMS (EI) *m/z* 499 (M^+); HRMS (EI) calcd for $C_{25}H_{41}N_5O_2Si_2$ 499.2799, found: 499.2803. Anal. Calcd for $C_{25}H_{41}N_5O_2Si_2 \cdot 1/2H_2O$: C, 59.01; H, 8.32; N, 13.76. Found: C, 59.19; H, 8.17; N,13.76.

6-*N*-Benzoyl-9-[3,5-bis(*tert*-butyldimethylsilanyloxymethyl)phenyl]adenine (20). A mixture of **19** (0.87 g, 1.73 mmol) and BzCl (0.24 mL, 2.08 mmol) in pyridine (9 mL) was stirred at room temperature. After 2 h, BzCl (40 μL, 0.35 mmol) was further added to the mixture. After 1 h, the mixture was partitioned between EtOAc and aqueous $NaHCO_3$ (saturated). The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (SiO_2, 20−100% EtOAc in hexane) to give **20** (0.506 g, 49% as a white solid): mp 210−212 °C; ^{1}H NMR ($CDCl_3$) δ 9.08 (s, 1H), 8.87 (s, 1H), 8.31 (s, 1H), 8.07−7.40 (m, 8H), 4.86 (s, 4H), 0.97 (s, 18H), 0.15 (s, 12H); ^{13}C NMR ($CDCl_3$) δ 165.6, 154.3, 150.8, 144.9, 143.0, 135.3, 134.6, 133.8, 129.9, 128.8, 124.5, 124.1, 120.3, 65.3, 26.9, 19.4, −4.3; LRMS (EI) *m/z* 603 (M^+); HRMS (EI) calcd for $C_{32}H_{45}N_5O_3Si_2$ 603.3061, found 603.3068. Anal. Calcd for $C_{32}H_{45}N_5O_3Si_2 \cdot 1/2H_2O$: C, 62.71; H, 7.56; N, 11.43. Found: C, 62.80; H, 7.55; N, 11.39.

6-*N*-Benzoyl-9-[3-(*tert*-butyldimethylsilanyloxymethyl)-5-(hydroxymethyl)phenyl]adenine (21). A mixture of **20** (0.215 g, 0.30 mmol), TBAF (1 M in THF, 0.15 mL, 0.15 mmol), THF (2.8 mL), and CH_2Cl_2 (2.8 mL) was stirred at room temperature. After 4 h, TBAF (1 M in THF, 0.3 mL, 0.3 mmol) was further added to the solution. After 4 h, the mixture was partitioned between $CHCl_3$ and aqueous $NaHCO_3$ (saturated). The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (SiO_2, 2.5% MeOH in $CHCl_3$) to give **20** (96 mg, 44%) and **21** (92 mg, 53% as a white solid): mp 184−186 °C; ^{1}H NMR ($CDCl_3$) δ 9.10 (s, 1H), 8.84 (s, 1H), 8.29 (s, 1H), 8.05−7.39 (m, 8H), 5.46 (s, 1H), 4.84 (m, 4H), 0.95 (s, 9H), 0.13 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 164.6, 153.3, 149.8, 144.3, 143.2, 141.9, 134.5, 132.9, 128.9, 127.9, 124.0, 123.4, 120.1, 120.0, 64.5, 64.2, 25.9, 18.4, −5.3; LRMS (EI) *m/z* 489 (M^+); HRMS (EI) calcd for $C_{26}H_{31}N_5O_3Si$ 489.2196, found 489.2207. Anal. Calcd for $C_{26}H_{31}N_5O_3Si \cdot 1/2H_2O$: C, 62.62; H, 6.47; N, 14.04. Found: C, 62.70; H, 6.45; N, 13.57.

6-*N*-Benzoyl-9-[3-(4,4′-dimethoxytrityloxymethyl)-5-(hydroxymethyl)phenyl]adenine (22). A mixture of **21** (0.38 g, 0.78 mmol), DMTrCl (0.53 g, 1.56 mmol), and DMAP (5 mg, 40 μmol) in pyridine (4 mL) was stirred at room temperature for 16 h. The mixture was partitioned between $CHCl_3$ and aqueous $NaHCO_3$ (saturated). The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was dissolved in THF (4 mL). TBAF (1 M in THF, 1.56 mL, 1.56 mmol) was added to the solution, and the whole was stirred at room temperature for 17 h. The mixture was partitioned between $CHCl_3$ and aqueous $NaHCO_3$ (saturated). The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (SiO_2, 1−2% MeOH in $CHCl_3$) to give **22** (0.45 g, 84% as a white solid): ^{1}H NMR ($CDCl_3$) δ 9.03 (s, 1H), 8.89 (s, 1H), 8.31 (s, 1H), 8.06 (d, 2H, J = 7.6), 7.69−6.82 (m, 19H), 4.84 (d, 2H, J = 5.6), 4.33 (s, 2H), 3.79 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 164.7, 158.6, 153.2, 151.8, 149.9, 149.5, 144.7, 143.3, 142.1, 142.0, 135.9, 134.4, 133.6, 132.8, 130.0, 128.9, 128.1, 128.0, 127.9, 126.9, 125.0, 123.3, 120.9, 120.3, 64.9, 64.4, 55.2, 53.6, 39.0, 20.7, 14.0; LRMS (FAB) *m/z* 678 (MH^+); HRMS (FAB) calcd for $C_{41}H_{36}N_5O_5$ 678.2716, found 678.2711.

9-[3,5-Bis(hydroxymethyl)phenyl]adenine (3). A mixture of **16** (0.764 g, 2.72 mmol), $(EtO)_3CH$ (3.4 mL), and Ac_2O (3.4 mL) was refluxed for 16 h. The solvent was evaporated in vacuo. The resulting residue was treated with methanolic ammonia (100 mL, saturated at 0 °C) in a steel sealed tube at 90 °C for 16 h. After the tube was cooled to room temperature then degassed, the solvent was evaporated in vacuo. The resulting residue was purified by column chromatograph (SiO_2, 1% MeOH in $CHCl_3$) to give **3** (0.265 g, 36% as a white solid): mp 244−246 °C; UV λmax (H_2O) 213 nm, 243 nm, 270 nm; ^{1}H NMR (DMSO-d_6) δ 8.53 (s, 1H), 8.20 (s, 1H), 7.66 (s, 2H), 7.37 (m, 3H), 5.35 (t, 2H, J = 5.2), 4.58 (d, 4H, J = 5.2); ^{13}C NMR (DMSO-d_6) δ 156.3, 153.1, 149.2, 144.0, 139.6, 134.8, 123.2, 119.2, 119.1, 62.5; LRMS (EI) *m/z* 271 (M^+); HRMS (EI) calcd for $C_{13}H_{13}N_5O_2$ 271.1069, found: 271.1075. Anal. Calcd for $C_{13}H_{13}N_5O_2 \cdot 1/10H_2O$: C, 57.18; H, 4.87; N, 25.65. Found: C, 57.16; H, 4.73; N, 25.52.

2-Amino-4-[3,5-bis(hydroxymethy)anilino]-6-hydroxy-5-phenylazopyrimidine (24). A mixture of **5** (0.50 g, 3.30 mmol) and 2-amino-4-chloro-6-hydroxy-5-phenylazopyrimidine (**23**)[10] (1.24 g, 4.95 mmol) in EtOH (9 mL) was refluxed. After 7 h, the mixture was allowed to stand overnight in the refrigerator. The product was filtered and washed with Et_2O to give **24** (0.84 g, 70%): mp 210−212 °C; ^{1}H NMR (DMSO-d_6) δ 7.86 (s, 1H), 7.51−7.16 (m, 8H), 5.26 (s, 2H), 4.53 (s, 4H), 3.33 (s, 2H); LRMS (EI) *m/z* 366 (M^+); HRMS (EI) calcd for $C_{18}H_{18}O_3N_6$ 366.1440, found 366.1449.

2-Amino-4-[3,5-bis(*tert*-butyldimethylsilanyloxymethyl)-anilino]-6-hydroxy-5-phenylazopyrimidine (25). A mixture of **24** (0.50 g, 1.37 mmol), TBDMSCl (0.62 g, 4.10 mmol), and imidazole (0.56 g, 8.20 mmol) in DMF (3 mL) was stirred at room temperature for 18 h. The mixture was partitioned between EtOAc and H_2O. The organic layer was washed with aqueous $NaHCO_3$ (saturated) and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (SiO_2, 4% MeOH in $CHCl_3$) to give **25** (0.804 g, 99%): mp 216−218 °C; ^{1}H NMR ($CDCl_3$) δ 7.79−7.06 (m, 8H), 4.78 (d, 4H, J = 7.6), 0.98 (s, 18H), 0.15 (s, 12H); LRMS (EI) *m/z* 594 (M^+); HRMS (EI) calcd for $C_{30}H_{46}N_6O_3Si_2$ 594.3170, found 594.3185.

9-[3,5-Bis(*tert*-butyldimethylsilanyloxymethyl)phenyl]-2-*N*-[(dimethylamino)methylene]guanine (26). A mixture of **25** (5.05 g, 8.50 mmol), Zn powder (5.53 g, 84.6 mmol), AcOH (6.4 mL), H_2O (63 mL), EtOH (72 mL), and THF (72 mL) was refluxed for 1 h. After the excess Zn was filtered off, the solvent was partitioned between EtOAc and H_2O. The organic layer was washed with aqueous $NaHCO_3$ (saturated) and brine, dried (Na_2SO_4), and evaporated in vacuo. The resulting residue was mixed with *N,N*-dimethylformamide dimethyl acetal (14.5 mL, 0.11 mmol) and DMF (125 mL), and the whole was stirred at room temperature for 15 h. The solvent was evaporated in vacuo. The resulting residue was mixed with $(EtO)_3CH$ (19 mL, 113 mmol) and Ac_2O (19 mL, 198 mmol), and the whole was stirred at room temperature for 7 h. The mixture was partitioned between EtOAc and H_2O. The organic layer was washed with aqueous $NaHCO_3$ (saturated) and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (SiO_2, 2.5% MeOH in $CHCl_3$) to give **26** (3.70 g, 76%): mp 89−90 °C; ^{1}H NMR ($CDCl_3$) δ 8.64 (s, 1H), 8.51 (s, 1H), 7.80 (s, 1H), 7.42 (s, 2H), 7.34 (s, 1H), 4.81 (s, 4H), 3.12 (s, 3H), 3.09 (s, 3H), 0.95 (s, 18H), 0.12 (s, 12H); ^{13}C NMR ($CDCl_3$) δ 158.2, 158.0, 156.8, 150.2, 143.3, 137.8, 135.0, 122.8, 121.0, 120.1, 64.4, 41.3, 35.2, 25.9, 18.4, −5.3; LRMS (EI) *m/z* 570 (M^+); HRMS (EI) calcd for $C_{28}H_{46}N_6O_3Si_2$

570.3170, found 570.3164. Anal. Calcd for $C_{28}H_{46}N_6O_3Si_2 \cdot 1/2H_2O$: C, 57.99; H, 8.17; N, 14.49. Found: C, 58.34; H, 8.33; N, 14.15.

9-[3-(*tert*-Butyldimethylsilanyloxymethyl)-5-(hydroxymethyl)phenyl]-2-*N*-[(dimethylamino)methylene]-guanine (27). A mixture of **26** (0.204 g, 0.36 mmol) and TBAF (1 M in THF, 0.18 mL, 0.18 mmol) in THF (5 mL) was stirred at room temperature for 30 min. The mixture was partitioned between $CHCl_3$ and aqueous $NaHCO_3$ (saturated). The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (SiO_2, 3−5% MeOH in $CHCl_3$) to give **27** (95 mg, 59% as a white solid): mp 216−217 °C; ^{1}H NMR ($CDCl_3$) δ 8.61 (s, 1H), 8.53 (s, 1H), 7.83 (s, 1H), 7.61 (s, 1H), 7.52 (s, 1H), 7.34 (s, 1H), 4.81 (s, 4H), 3.13 (s, 3H), 3.07 (s, 3H), 0.95 (s, 9H), 0.12 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 158.2, 156.9, 150.2, 143.4, 137.4, 135.2, 123.3, 120.8, 120.7, 119.4, 64.4, 64.3, 41.5, 35.0, 25.9, 18.4, −5.3; LRMS (FAB) *m/z* 457 (MH^+); HRMS (FAB) calcd for $C_{22}H_{33}N_6O_3Si_1$ 457.2305, found 457.2393.

9-[3-(4,4′-Dimethoxytrityloxymethyl)-5-(hydroxymethyl)phenyl]-2-*N*-[(dimethylamino)methylene]guanine (28). A mixture of **27** (0.95 g, 2.09 mmol), DMTrCl (1.42 g, 4.18 mmol), and DMAP (13 mg, 0.11 mmol) in pyridine (10 mL) was stirred at room temperature for 18 h. The mixture was partitioned between $CHCl_3$ and aqueous $NaHCO_3$ (saturated). The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was dissolved in THF (11 mL). TBAF (1 M in THF, 4.18 mL, 4.18 mmol) was added to the solution, and the mixture was stirred at room temperature for 5 h. The mixture was partitioned between $CHCl_3$ and aqueous $NaHCO_3$ (saturated). The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (SiO_2, 3−5% MeOH in $CHCl_3$) to give **28** (1.16 g, 86% as a white solid): ^{1}H NMR ($CDCl_3$) δ 8.78 (s, 1H), 8.45 (s, 1H), 7.89−6.82 (m, 17H), 4.78 (s, 2H), 4.25 (s, 2H), 3.78 (s, 6H), 2.92 (s, 3H), 2.53 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 158.6, 158.1, 158.0, 156.8, 150.2, 144.9, 143.1, 141.2, 137.3, 136.0, 135.4, 129.9, 128.0, 126.9, 124.0, 121.0, 120.5, 120.1, 113.2, 86.5, 64.9, 64.4, 55.2, 40.7, 35.0; LRMS (FAB) *m/z* 645 (MH^+); HRMS (FAB) calcd for $C_{37}H_{37}N_6O_5$ 645.2747, found 645.2819. Anal. Calcd for $C_{37}H_{36}N_6O_5 \cdot 5/4H_2O$: C, 66.99; H, 5.99; N, 12.34. Found: C, 66.83; H, 5.73; N, 12.09.

9-[3,5-Bis(hydroxymethyl)phenyl]guanine (4). A mixture of **24** (0.70 g, 1.91 mmol), Zn powder (1.25 g, 19.1 mmol), AcOH (1.4 mL), H_2O (14 mL), EtOH (16 mL), and THF (16 mL) was refluxed for 30 min. After the excess Zn was filtered off, the solvent was evaporated in vacuo. The resulting residue was mixed with $(EtO)_3CH$ (26 mL, 157 mmol), DMF (13 mL), and 1 M HCl (1.2 mL), and the whole was stirred at room temperature for 18 h. The solvent was evaporated in vacuo. The resulting residue was dissolved in 0.5 M HCl (36 mL), and the whole was stirred at room temperature for 1 h. The product was filtered and recrystallized from MeOH to give **4** (0.27 g, 25%): UV λ_{max} (H_2O) 210 nm, 242 nm, 262 nm; ^{1}H NMR (DMSO-d_6) δ 10.67 (s, 1H), 7.93 (s, 1H), 7.42 (s, 2H), 7.35 (s, 1H), 6.48 (s, 2H), 5.31 (t, 2H, J = 5.4), 4.55 (d, 4H, J = 5.4); ^{13}C NMR (DMSO-d_6) δ 157.5, 154.1, 151.5, 144.2, 137.4, 135.0, 124.2, 120.8, 117.3, 62.8; LRMS (FAB) *m/z* 288 (MH^+); HRMS (FAB) calcd for $C_{13}H_{14}N_5O_3$ 288.1097, found 288.1101. Anal. Calcd for $C_{13}H_{13}N_5O_3 \cdot 1/7H_2O$: C, 53.87; H, 4.62; N, 24.16. Found: C, 54.11; H, 4.64; N, 24.07.

1-[3-[[(2-Cyanoethoxy)(*N,N*-diisopropylamino)phosphinyl]oxymethyl]-5-(4,4′-dimethoxytrityloxymethyl)-phenyl]uracil (29). Compound **8** (0.80 g, 1.45 mmol) was dissolved in THF (9.7 mL) containing *N,N*-diisopropylethylamine (1.46 mL, 8.72 mmol). Chloro(2-cyanoethoxy)(*N,N*-diisopropylamino)phosphine (0.65 mL, 2.9 mmol) was added to the solution, and the mixture was stirred at room temperature for 1 h. Aqueous $NaHCO_3$ (saturated) and $CHCl_3$ were added to the mixture, and the separated organic layer was washed with aqueous $NaHCO_3$ (saturated) and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (a neutralized SiO_2, EtOAc) to give **29** (0.88 g, 81%): ^{31}P NMR ($CDCl_3$) δ 148.9.

4-*N*-Benzoyl-1-[3-[[(2-cyanoethoxy)(*N,N*-diisopropylamino)phosphinyl]oxymethyl]-5-(4,4′-dimethoxytrityloxymethyl)phenyl]cytosine (30). Compound **12** (0.66 g, 1.01 mmol) was phosphitylated as described in the preparation of **29** to give **30** (0.83 g, 96%): ^{31}P NMR ($CDCl_3$) δ 149.4.

6-*N*-Benzoyl-9-[3-[[(2-cyanoethoxy)(*N,N*-diisopropylamino)phosphinyl]oxymethyl]-5-(4,4′-dimethoxytrityloxymethyl)phenyl]adenine (31). Compound **22** (0.45 g, 0.66 mmol) was phosphitylated as described in the preparation of **29** to give **31** (0.51 g, 88%): ^{31}P NMR ($CDCl_3$) δ 149.4.

9-[3-[[(2-Cyanoethoxy)(*N,N*-diisopropylamino)phosphinyl]oxymethyl]-5-(4,4′-dimethoxytrityloxymethyl)-phenyl]-2-*N*-[(dimethylamino)methylene]guanine (32). Compound **28** (0.78 g, 1.21 mmol) was phosphitylated as described in the preparation of **29** to give **32** (0.62 g, 60%): ^{31}P NMR ($CDCl_3$) δ 149.3.

Solid Support Synthesis. A mixture of **8** (0.15 g, 0.27 mmol), succinic anhydride (90 mg, 0.90 mmol), and DMAP (33 mg, 0.27 mmol) in pyridine (3 mL) was stirred for 72 h at room temperature. The solution was partitioned between $CHCl_3$ and H_2O, and the organic layer was washed with H_2O and brine. The separated organic phase was dried (Na_2SO_4) and concentrated to give the corresponding succinate. Aminopropyl controlled pore glass (0.50 g, 45 μmol) was added to a solution of the succinate and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (63 mg, 0.33 mmol) in DMF (8 mL), and the mixture was kept for 72 h at room temperature. After the resin was washed with pyridine, a capping solution (8 mL, 0.1 M DMAP in pyridine:Ac_2O = 9:1, v/v) was added and the whole mixture was kept for 16 h at room temperature. The resin was washed with MeOH and acetone, and dried in vacuo. The amount of loaded compound **8** to the solid support was 67 μmol/g from calculation of released dimethoxytrityl cation by a solution of 70% $HClO_4$:EtOH (3:2, v/v). In a similar manner, the solid supports with **12**, **22**, and **28** were obtained in 82, 54, and 70 μmol/g loading amounts, respectively.

X-ray Crystallography. Crystal data for **1**: Colorless plate (0.51 × 0.40 × 0.03 mm); $C_{12}H_{12}N_2O_4$, monoclinic space group *Cc*, Z = 4. a = 4.354(4) Å, b = 22.74(2) Å, c = 12.12(1) Å, β = 105.022(8)°. X-ray diffraction measurements were carried out on a diffractometer with a Mercury CCD area detector (Mo−Kα λ = 0.71069 Å). Of the 4743 measured reflections (θ < 27.48°), 1330 were unique (R_{int} = 0.036). All calculations were performed using the teXsan crystallographic software package. The structure was solved by the direct methods SIR92. The final least-squares refinement included non-hydrogen and hydrogen atoms with anisotropic and isotropic thermal parameters, respectively. The refinement converged at R_1 = 0.035 for 1093 reflections with $I > 2\sigma(I)$ and R_w = 0.068 for all reflections.

Oligomer Synthesis. The synthesis was carried out with a DNA/RNA synthesizer by the phosphoramidite method. For the incorporation of the analogues into the oligomers, a 0.12 M solution of each analogue phosphoramidite in THF with a coupling time of 15 min was used. Deprotection of the bases and phosphates was performed in concentrated NH_4OH at 55 °C for 16 h. The deprotected ONAs and 2′-*O*-Me-ONAs were purified by 20% PAGE containing 7 M urea to give the highly purified ONAs **7** (5), **8** (5), **9** (3), **10** (3), **11** (6), **12** (3), **13** (3), **14** (12), **15** (6), **18** (4), **19** (7), **20** (2), **21** (5), 2′-*O*-Me-ONAs **23** (20), and **24** (7). The yields are indicated in parentheses as OD units at 260 nm starting from 1 μmol scale.

MALDI-TOF/MS Analyses of Oligomers. Spectra were obtained with a time-of-flight mass spectrometer. ONA **7**: calculated mass, 3828.7; observed mass, 3828.2. ONA **8**: calculated mass, 3828.7; observed mass, 3831.4. ONA **9**: calculated mass, 3804.7; observed mass, 3807.5. ONA **10**: calculated mass, 3844.7; observed mass, 3845.7. ONA **11**: calculated mass, 3827.7; observed mass, 3830.2. ONA **12**:

calculated mass, 3874.8; observed mass, 3875.2. ONA **13**: calculated mass, 3784.6; observed mass, 3781.9. ONA **14**: calculated mass, 3936.9; observed mass, 3938.2. ONA **15**: calculated mass, 3660.4; observed mass, 3662.5. ONA **18**: calculated mass, 5186.4; observed mass, 5184.8. ONA **19**: calculated mass, 5220.4; observed mass, 5221.3. ONA **20**: calculated mass, 5226.5; observed mass, 5227.7. ONA **21**: calculated mass, 5232.4; observed mass, 5234.0. 2′-*O*-Me-ONA **23**: calculated mass, 5666.9; observed mass, 5659.6. 2′-*O*-Me-ONA **24**: calculated mass, 5646.9; observed mass, 5641.8.

Hyperchromicities and Extinction Coefficients of the Oligomers. Each oligomer (0.2 OD unit at 260 nm) was incubated with snake venom phosphodiesterase (1.0 unit), nuclease P1 (1.0 unit), and alkaline phosphatase (1.0 unit) in a buffer containing 100 mM Tris-HCl (pH 7.7) and 2 mM $MgCl_2$ (total 600 μL) at 37 °C for 48 h. Hyperchromicity of each oligomer was determined by comparing UV absorbances at 260 nm of the solutions before and after hydrolyses. The extinction coefficients (at 260 nm) of each oligomer was determined using the following equation: $\epsilon_{oligomer}$ = the sum of $\epsilon_{nucleoside}$/hyperchromicity. The extinction coefficient (at 260 nm) of the natural nucleosides used for calculations were as follows: dA, 15400; dC, 7300; dG, 11700; T, 8800. The extinction coefficients of the analogues at 260 nm were determined to be the following: $\mathbf{A^B}$, 18500; $\mathbf{C^B}$, 9050; $\mathbf{G^B}$, 15300; $\mathbf{U^B}$, 13100. The extinction coefficients of the natural ONs were calculated from those of mononucleotides and dinucleotides according to the nearest-neighbor approximation method.[18]

Thermal Denaturation Study and CD Spectroscopy. A solution containing the ONs in a buffer comprised of 10 mM sodium phosphate (pH 7.0) and 1.0 or 0.1 M NaCl was heated at 95 °C for 3 min, cooled gradually to an appropriate temperature, and then used for the thermal denaturation study. The thermal-induced transition of each mixture was monitored at 260 nm with a spectrophotometer. The sample temperature was increased by 0.5 °C/min. CD spectra were measured by a spectropolarimeter. Samples for CD spectroscopy were prepared by the same procedure used in the thermal denaturation study, and spectra were measured at 10 °C. The molar ellipticitiy was calculated from the equation $[\theta] = /cL$, where θ is the relative intensity, c the sample concentration, and L the cell path length in centimeters.

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Supporting Information Available: Crystallographic information file for the compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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