A New Method for the Synthesis of Oligodeoxyribonucleotides Containing 4-*N*-Alkoxycarbonyldeoxycytidine Derivatives and Their Hybridization Properties

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Oligodeoxyribonucleotides incorporating 4-*N*-alkoxycarbonyldeoxycytidine derivatives were synthesized on polystyrene-type ArgoPore resins having a new benzyloxy(diisopropyl)silyl linker, by use of $ZnBr_2$ as the detritylating agent. The first 3′-terminal thymidine could be attached to the resin by successive in situ reactions of 5′-*O*-DMTr-thymidine with diisopropylsilanediyl ditriflate and an ArgoPore resin containing hydroxyl groups. The use of this new silanediyl-type linker allowed release of the DNA chain from the resin by treatment with TBAF under neutral conditions. The T_m experiments apparently showed that incorporation of 4-*N*-alkoxycarbonyldeoxycytidines into DNA strands resulted in higher hybridization affinity with the complementary DNA strands than that of 4-*N*-acyldeoxycytidines. In addition, comparable T_m studies using oligodeoxyribonucleotides incorporating acyl (RC(O)−) groups and alkoxyacyl ($RO(CH_2)_nC(O)$−) groups having the same chain length show that the latter tend to exhibit higher T_m values than the former. It turned out that 4-*N*-alkoxycarbonyldeoxycytidines can form base pairs not only with deoxyguanosine but also with deoxyadenosine. Based on the ab initio calculations of the hydrogen bond energies of the possible base pairs formed between 4-*N*-methoxycarbonyl-1-methylcytosine and 9-methyladenine and the NMR analysis of the base-pairs of ^{15}N-labeled 4-*N*-alkoxycarbonyldeoxycytidines with deoxyadenosine derivatives, we conclude that the base pair involves two unique hydrogen bonds between the cytosyl 4-NH group and the adenyl N^1 atom and between the O atom of the ester group and the adenyl 6-NH group.

Introduction

N-Acylated nucleosides, such as 4-*N*-acetylcytidine (C^{ac})[1−7] and *N*-threonylcarbamoyladenosine (t6A),[8,9] are naturally occurring modified nucleosides found in tRNA and rRNA. It is known that C^{ac} exists at the first letter of the anticodon of some tRNAs and selectively forms the Watson−Crick-type base pair with guanosine.[10] Recently, we have reported the duplex stability of oligodeoxyribonucleotides having 4-*N*-acyldeoxycytidine derivatives.[11] Our results showed that acetylation of the 4-*N*-amino group of deoxycytidine stabilizes DNA duplexes, and the base recognition ability of this modified base has the same tendency as deoxycytidine. A variety of functional molecules such as biotin and fluorescein for DNA/RNA labeling and metal chelate complexes for DNA/RNA scission have long been introduced into the side chains at the 5-position of the cytosine base via amide bonds. Therefore, 4-*N*-acyldeoxycytidine derivatives would be more straightforward carriers of these functional groups that can be easily introduced into the 4-amino group by simple acylation. However, with an increase of the length of the side chain of the acyl group attached to the 4-*N*-amino group of deoxycytidine, DNA duplexes destabilize markedly because their increased hydrophobicity disturbs the hydrogen-bond structure around the major groove.[12] This kind of destabilization effect was also reported by use of *N*-alkylated deoxycytidines by Thong et al.[13] To stabilize DNA duplexes having a long side chain via an amide bond without disturbance of the hydration structure[14] around the major groove, we introduced more hydrophilic oxygen atoms into the alkyl side chain of the *N*-acyl group. In this paper, we report the duplex stability and base-recognition ability of oligodeoxyribonucleotides containing various 4-*N*-alkoxy-

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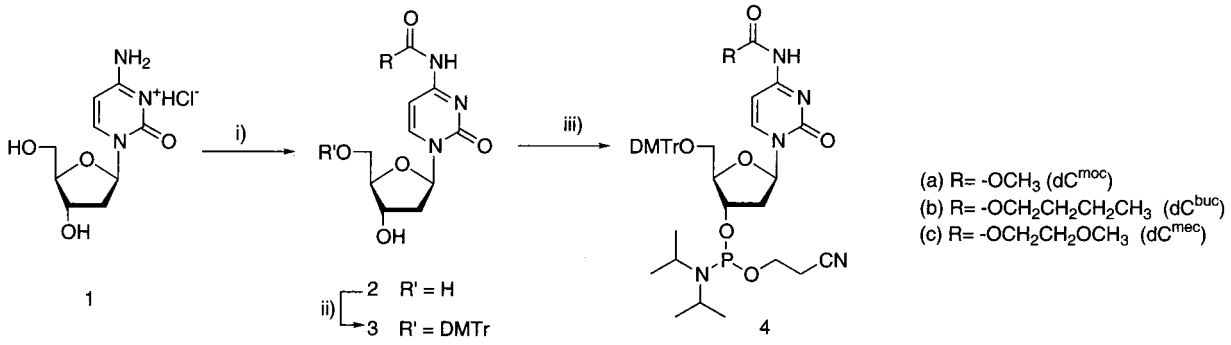
Scheme 1[a]



[a] Key: (i) (A) excess HMDS, CH_3CN, 60 °C, 30 min, (B) 1.5 equiv of chloroformate, 1.5 equiv of pyridine, CH_2Cl_2, 30 min, (C) concNH_3, 30 min; (ii) 1.2 equiv of DMTrCl, pyridine, rt, 3 h; (iii) 1.8 equiv of diisopropylethylamine, 1.5 equiv of chloro(2-cyanoethoxy)(*N*,*N*-diisopropylamino)phosphine, CH_2Cl_2, rt 1 h.

carbonyldeoxycytidine and 4-*N*-acyldeoxycytidine derivatives throughout T_m and ^{1}H NMR experiments, which suggests that the 4-*N*-alkoxycarbonyldeoxycytidine derivatives can form base pairs with not only deoxyguanosine but also deoxyadenosine.

Results and Discussion

Synthesis of Phosphoramidite Units. 4-*N*-Alkoxycarbonyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxycytidine-3′-*O*-phosphoramidite derivatives **4a**−**c** were synthesized from 2′-deoxycytidine (**1**), as described in Scheme 1. These alkoxycarbonyl groups were selectively introduced by the use of the corresponding alkyl chloroformate derivatives into the 4-*N*-amino function of deoxycytidine derivatives by a transient protection method using hexamethyldisilazane. Deprotection of the trimethylsilyl groups attached to the hydroxyl functions by treatment with ammonia gave 4-*N*-alkoxycarbonyldeoxycytidine derivatives **2a**−**c** as crystalline materials. 4-*N*-alkoxycarbonyl-5′-*O*-(4,4′-dimethoxytrityl)deoxycytidine derivatives **3a**−**c** were conveniently synthesized in high yields by in situ treatment of **2a**−**c** with DMTrCl. Compounds **3a**−**c** were converted to compounds **4a**−**c** by phosphitylation in the usual way.[15]

Synthesis of Nucleoside-Loaded Solid Supports. Recently, Fraser *et al.*[16] have reported a new diisopropylsilanediyl linker that can release oligodeoxyribonucleotides from solid supports by treatment with TBAF under neutral conditions. However, this linker proved to be partially lost during the acid treatment prescribed for removal of the DMTr group. In addition, Fraser's method required a four-step reaction for introduction of DMTrT into the solid support. Therefore, we made efforts to overcome these synthetic problems. To facilitate introduction of DMTrT into a resin, we used a polystyrene-type ArgoPore resin having hydroxyl functions, which have recently been used in the field of peptide synthesis.[17] DMTrT was linked with this resin in a more straightforward way, as shown in Scheme 2. Reaction of DMTrT (**5**) with diisopropylsilanediyl ditriflate in the presence of DBU gave a 3′-*O*-silylated species, which in turn was allowed to react with the hydroxyl group of the ArgoPore resin. After capping of unreacted hydroxyl functions with Ac_2O in the presence of DMAP, the DMTrT-loaded resin (**6**: 51.6 μmol/g) was obtained. When this resin was treated with 1 M TBAF−AcOH in THF for 1 h, 90% of the nucleoside was released from the solid support, as evidenced by the DMTr assay. Since partial cleavage of the silanediyl linker during the treatment with 3.0% trichloroacetic acid in CH_2Cl_2[18] was observed, we employed 1 M zinc bromide in CH_2Cl_2−*i*-PrOH (85/15, v/v).[12,19] This reagent did not cause undesired cleavage of the linker.

Synthesis, Purification, and Characterization of Oligodeoxyribonucleotides Containing Modified Bases. Solid-phase synthesis of oligodeoxyribonucleotides was carried out by use of the standard protocol. The usual chain elongation followed by the TBAF treatment gave oligodeoxyribonucleotides (**7a**−**c**, **8**), as shown in Scheme 2. The product was purified by anion-exchange HPLC and analyzed by reversed-phase HPLC. The isolated yields of the 13mers containing one or five modified nucleosides were 20−29%, as shown in Scheme 2. The structures of the purified products were confirmed by MALDI-TOF mass. The ratios of T and dC* (the dC* is a modified dC) in these modified DNA oligomers were confirmed by enzymatic digestion with snake venom phosphodiesterase and calf intestinal alkaline phosphatase. As reference materials, oligodeoxyribonucleotides d(T_6XT_6) containing 4-*N*-propionyldeoxycytidine (dC^{pr}, **7d**), 4-*N*-hexanoyldeoxycytidine (dC^{hex}, **7e**), and 4-*N*-(4-methoxybutyryl)-deoxycytidine (dC^{meb}, **7f**) were also synthesized by the method previously reported by us where a phthaloyl linker[11] was used for the polymer-supported synthesis.

Hybridization Property of Oligodeoxynucleotides Containing Modified Bases. The thermal stability of DNA duplexes d(T_6XT_6)/d(A_6GA_6) containing a modified nucleoside was investigated. The T_m values of DNA duplexes in phosphate buffer (pH 7.0) containing 1.0 M NaCl are listed in Table 1. Interestingly, the oligonucleotide [d($T_6C^{moc}T_6$)] (Table 1, entry 3) having a 4-*N*-methoxycarbonyldeoxycytidine formed a stable duplex with the complementary strand d(A_6GA_6) and had a higher T_m value (+1.0 °C) than the unmodified d(T_6CT_6). An oligodeoxyribonucleotide (**8**) [d($T_3C^{moc}TC^{moc}C^{moc}T_2C^{moc}$-

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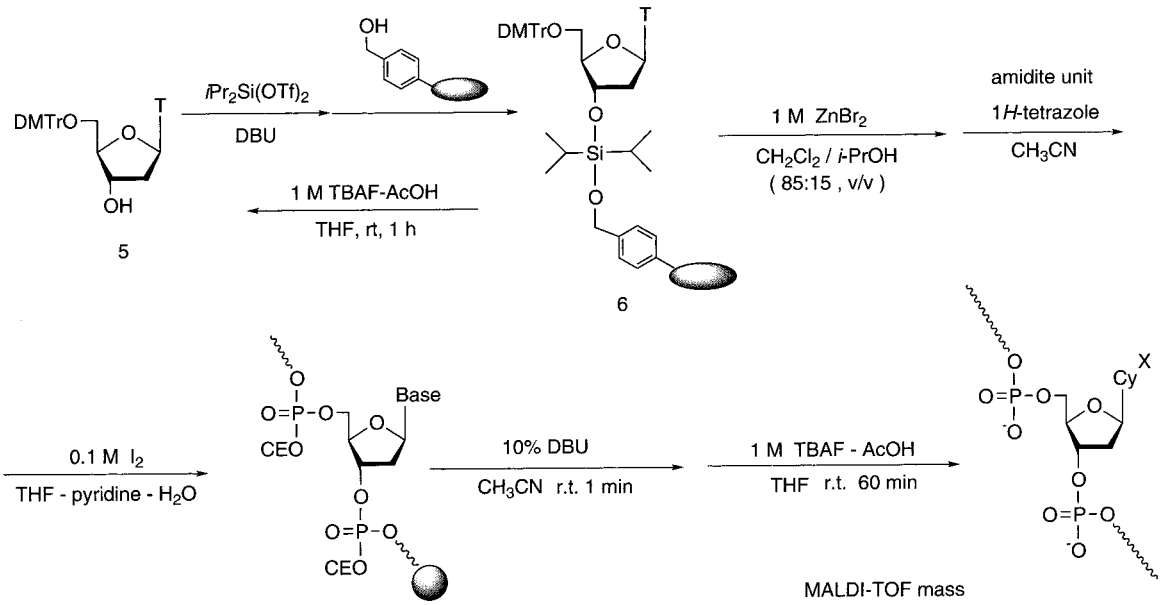
Scheme 2



		isolated yield	MALDI-TOF mass molecular	calcd	found
d(TTTTTTC^{moc}TTTTTT)	7a	20%	$C_{131}H_{171}N_{27}O_{90}P_{12}^-$	3934.6	3934.3
d(TTTTTTC^{buc}TTTTTT)	7b	29%	$C_{134}H_{177}N_{27}O_{90}P_{12}^-$	3975.7	3974.4
d(TTTTTTC^{mec}TTTTTT)	7c	24%	$C_{133}H_{175}N_{27}O_{91}P_{12}^-$	3976.7	3976.6
d(TTTC^{moc}TC^{moc}C^{moc}TTC^{moc}TC^{moc}T)	8	22%	$C_{135}H_{175}N_{31}O_{94}P_{12}^-$	4105.7	4104.7

Table 1. Melting Temperatures at 260 nm of a Natural Duplex and Those Containing a Modified C^{X}[a]

entry	5'-d (T T T T T T C^{X}T T T T T T) 3' / 3'-d (A A A A A A G A A A A A A) 5' X	abrev.	side chain length	number of oxygen	Tm(°C)	ΔTm1 (°C)	ΔTm2 (°C)
1	—	—	—	—	41.2	—	—
2	$CH_3CH_2C(O)$ (**7d**)	pr	2	0	40.7	-0.5	—
3	CH_3–O–C(O) (**7a**)	moc	2	1	42.2	+1.0	+1.5
4	$CH_3CH_2CH_2CH_2CH_2C(O)$ (**7e**)	hex	5	0	38.4	-2.8	—
5	CH_3–O–$CH_2CH_2CH_2C(O)$ (**7f**)	mob	5	1	40.0	-1.2	+1.6
6	$CH_3CH_2CH_2CH_2OC(O)$ (**7b**)	buc	5	1	39.8	-1.4	+1.4
7	CH_3–O–$CH_2CH_2OC(O)$ (**7c**)	mec	5	2	39.7	-1.5	+1.3
	5'-d (T T T C^{X}T C^{X}C^{X}T T C^{X}T C^{X}T) 3' / 3'-d (A A A G A G G A A G A G A) 5'						
8	—		2	0	52.1	—	
9	CH_3–O–C(O) (**8**)	moc	2	1	61.1	+9.0	

[a] ΔT_{m1} is the difference in the T_m value between the duplex having the modified bases and that having natural bases. ΔT_{m2} is the difference in the T_m value between the duplex having an *N*-acylated cytosine and that having an *N*-alkoxycarbonyl cytosine.

TC^{moc}T)] (Table 1, entry 9), which has five 4-*N*-methoxycarbonyldeoxycytidines in the strand, had a much higher T_m value (+9.0 °C) when hybridized with the complementary strand by comparison with the unmodified d(T_3-CTC_2T_2CTCT) (Table 1, entry 8). On the basis of these results, it is apparently concluded that the dC^{moc}-dG base pair stabilizes the DNA duplex more than the natural dC−dG base pair to a degree of 1.8 °C/one modification. Moreover, compared with an oligonucleotide (Table 1, entry 2) containing a 4-*N*-propionyldeoxycytidine (dC^{pr}) with the same chain length as the methoxycarbonyl group (Table 1, entry 3), it turned out that incorporation of an oxygen atom to the neighboring α-position of the carbonyl group apparently increased the duplex stability. In the previous paper,[11] we reported that incorporation of 4-*N*-acetyldeoxycytidines into oligodeoxyribonucleotides resulted in higher hybridization affinity with complementary strands than the corresponding natural-type oligonucleotides. However, the stabilization effect was weakened by increasing the side chain length of the acyl group.[12] In this study, entries 4 and 6 of Table 1 showed the same tendency. These results suggested that the hydrophobicity of the side chain of the acyl group was partly responsible for the duplex destabilization, as shown in Table 1, entries 4 and 6. In the case of the duplexes of entries 4−7 (Table 1) which have longer side chain lengths, they have lower T_m values by 1.2−2.8 °C than the duplex of entry 1 (Table 1) because of their increased hydrophobicity, but the T_m values of the duplexes of entries 5−7 (Table 1) are higher than that of the duplex of entry 4 (Table 1). The duplexes of entries 5−7 (Table 1) have commonly one or two oxygen atoms in the side chain to keep the hydration structure in the major groove that is essential for stabilization of the DNA duplex structure. It is likely that this stabilization was caused by increase of the hydrophilicity of the side chain due to incorporation of the oxygen atom.

Base Recognition Ability of 4-*N*-Alkoxycarbonyldeoxycytidines. The T_m values of DNA duplexes having a mismatched base pair in X−Y are summarized in Table 2. The duplexes having amide-type, *N*-acylated deoxycytidine derivatives (Table 2, entries 2−4) were destabilized by the one-base mismatch to the same degree as the unmodified duplex d(T_6CT_6)/d(A_6GA_6) (Table 2, entry 1). These results are in agreement with the previous result.[11] The duplexes having urethane-type, *N*-acylated deoxycytidine derivatives (Table 2, entries 5−7) were considerably more stabilized than the natural duplex, when deoxyadenosine was located at the opposite position of 4-*N*-alkoxycarbonyl deoxycytidine derivatives.

Table 2. Melting Temperatures at 260 nm of a Natural Duplex and Those Containing a Modified C^X

5'-d(TTTTTTT C^XTTTTTTT)3' / 3'-d(AAAAAAA Y AAAAAAA)5'			Tm (°C)				
entry	X	abrev.	Y = G	Y = A	ΔTm (G - A)	Y = T	Y = C
1	—	—	41.2	28.6	12.6	27.3	20.5
2	$CH_3CH_2C(O)$ (**7d**)	pr	40.7	29.0	11.7	29.0	19.5
3	$CH_3CH_2CH_2CH_2CH_2C(O)$ (**7e**)	hex	38.4	28.6	9.8	26.9	21.1
4	CH_3–O–$CH_2CH_2CH_2C(O)$ (**7f**)	meb	40.0	28.6	11.4	27.6	18.5
5	CH_3–O–C(O) (**7a**)	moc	42.2	35.9	6.3	29.7	23.3
6	$CH_3CH_2CH_2CH_2OC(O)$ (**7b**)	buc	39.8	35.1	4.7	27.7	21.2
7	CH_3–O–$CH_2CH_2OC(O)$ (**7c**)	mec	39.7	34.3	5.4	28.0	21.1

As shown in Table 2, all duplexes having a T−C* mismatched base pair have T_m values between 27.3 and 29.7 °C. Acyl modification on the cytosine base did not essentially affect the stability of these mismatched duplexes regardless of the type of acyl groups. In the case of the duplexes having a C−C* mismatched base pair, there is a more varied range (18.5−23.3 °C) in the T_m values observed. Such variation did not influence the G-base recognition of dC* since the T_m values observed were too low.

More significant effects were observed by replacement of the dA−dC base pair with a dA−dC* base pair on the duplex stability. Compared with a series of amide-type *N*-acylated deoxycytidine derivatives, urethane-type deoxycytidine derivatives exhibited significant increases of the T_m values, as shown in entries 5−7 of Table 2. In the case of dC^{moc} having the MeOC(O) group (Table 2, entry 5), the T_m value increased up to 35.9 °C, which is 6.3 °C higher than that of dC (Table 2, entry 1). The most serious loss of the base recognition ability was observed in the case of dC^{buc} having a BuOC(O) group (Table 2, entry 6) . There is a difference of only 4.7 °C in the T_m values between the duplexes having a dC^{buc}−dG matched base pair and a dC^{buc}−dA mismatched base pair. The approach of the T_m values to a similar level is interesting with respect to studies of mutagenesis of DNA. It should be clarified if incorporation of dC^{buc} into DNA can cause DNA mutagenesis. Our results strongly suggested that there might be a possibility that a series of urethane-type *N*-acylated deoxycytidine derivatives could be used for such studies.

Estimation of Hydrogen Bonding Energies of Mismatched Base Pairs by Use of ab Initio Calculation. As possible factors to be discussed for duplex stabilization due to the C*−A mismatched base pair, the base-stacking mode, base-pairing mode, and hydrogen bonding energy between the C*−A base pair should be taken into consideration. To examine the most reasonable mode of the mismatched base pair, the energy difference between the structural isomers of 4-*N*-methoxycarbonyl-1-methylcytosine and the hydrogen bond energies of the possible base pairs formed between 4-*N*-methoxycarbonyl-1-methylcytosine (**9**) and 9-methyladenine (**10**) were estimated in the gas phase by using the Gaussian 98 package program.[20] Figure 2 shows three possible isomeric structures (**9a**−**c**) of 4-*N*-methoxy-1-methylcytosine. The structure **9b** is a rotamer of **9a** around O*−C−N4−C4, while the structure **9c** is a tautomer of **9a**. Table 3 shows their relative energies in gas ($\epsilon = 0$) and solution phases ($\epsilon = 40$ under the same circumstance as inside the DNA helix).[21] The standard structure of **9a** having the same orientation of the carbonyl oxygen as that of the crystal structure of 4-*N*-acetylcytidine[22] was found to be the most stable conformer throughout four kinds of calculations. In the gas phase, the difference in energy between **9a** and **9b** was very narrow at a higher level calculation (B3LYP/6-31++G**). To calculate the energies of **9a**, **9b**, and **9c** in the solution phase, the effect of a bulk solvent was simulated using the IPCM continuum reaction field[23] with the isodensity contour set to 0.0004 electrons per cubic bohr.[24] Consequently, **9a** was confirmed to be the most stable form, while the tautomer **9c** was the most unfavorable structure in the solution phase. These results strongly suggested that in both gas and solution phases 4-*N*-methoxy-1-methylcytosine exists in the form of **9a**.

Figure 2 shows three possible base-pairing modes **11a**−**c** between 4-*N*-methoxycarbonyl-1-methylcytosine **9** and 9-methyladenine **10**, which can form two hydrogen bonds without great deformation. In the case of hydrogen bonding energy calculation, the C^{moc}−A mismatched pair including the unfavorable imino tautomeric form **9c** was the most stable, and hydrogen bonding energy was estimated to be −8.28 kcal/mol, as shown in **11c** of Figure 2. The origin of the stabilization effect of this base pair was due to the markedly negatively charged 4-nitrogen atom with the Mulliken charge of −0.91 e and the positively charged (+0.56 e) proton of the NH group at position 3. The C^{moc}−A mismatched pair (**11a**) has a smaller hydrogen bonding energy (−6.34 kcal/mol) even though it includes the most favorable amino tautomer. The partial charge of the oxygen in the ester group was −0.42 e and decreased by −0.07 e compared with the partial charge in the monomer **9a** in Figure 2 (data not shown). This means that the oxygen atom in the ester group of **9a** can take part in hydrogen bonding with the amino proton of A.[26] The hydrogen bonding energy (−5.52 kcal/mol) of the C^{moc}−A mismatched pair **11b** was slightly lower than that of the **11a** type. The energy differences of three types of mismatched base pairs were within 2.8 kcal/mol. Therefore, it was suggested that the rotation of the amide bond and the tautomerism between the amino and imino forms would be responsible for stabilization of C^{moc}−A mismatched base pair. To estimate the energy of the mismatched base pair exactly, a higher level calculation and solvation calculation using explicit water molecules were required to reproduce the environment of the DNA helix.

^{1}H NMR Studies of Base Pairs Formed between 3′,5′-*O*-TBDMS-4-*N*-methoxycarbonyldeoxycyti-

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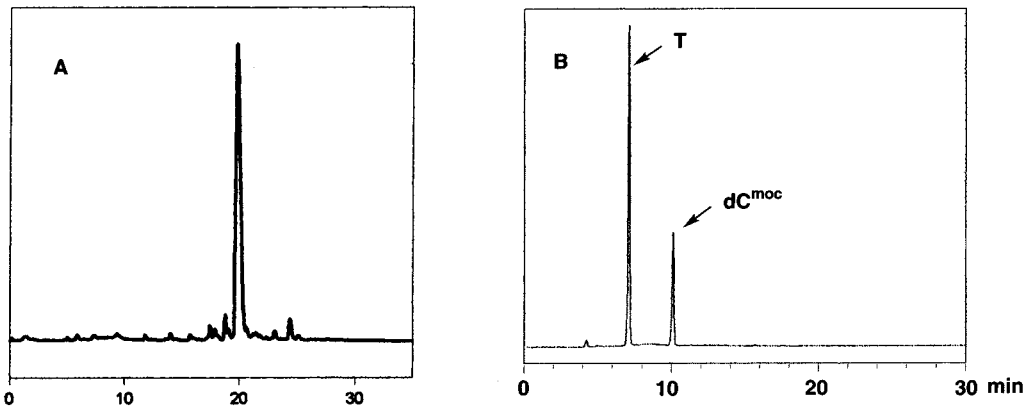

Figure 1. Anion-exchange HPLC profile of **8** (A) and reversed-phase HPLC profile of the mixture obtained by enzymatic digestion of **8** (B).

dine and 3′,5′-*O*-TBDMS-deoxyadenosine. NMR measurements were performed at 223 K in deuterated chloroform to confirm if 4-*N*-methoxycarbonyl-3′,5′-*O*-bis-(*tert*-butyldimethylsilyl)deoxycytidine (**12**) can form a base pair with 3′,5′-*O*-bis(*tert*-butyldimethylsilyl)deoxyadenosine (**14**) via hydrogen bonds.

Figure 3A shows the ^{1}H NMR spectra of **12** at various temperatures. The chemical shift of unexchangeable protons of compound **12** did not change at any temperature except for the 6H proton, which slightly changed from 8.34 ppm at 313 K to 8.59 ppm at 223 K. This change of 6H is also observed in the case of 3′,5′-*O*-bis-(*tert*-butyldimethylsilyl)deoxycytidine (**13**) (data not shown). The 5H vinyl proton at ca. 7.2 ppm did not move. This implies that an intramolecular hydrogen bond exists in the range between −50 and +40 °C. This chemical shift of 7.2 ppm is remarkably moved to the downfield from 5.6 ppm, which is usual for *N*-unprotected cytidine derivatives.[12,22,26] Therefore, this NMR study revealed this compound **12** exists in the form of **9a**, as suggested by the *ab initio* calculation. Furthermore, the small mobility of the chemical shift of the amide proton of **12** from 7.42 ppm at 313 K to 7.98 ppm at 223 K implied that compound **12** did not form a self-assembled base pair in $CDCl_3$ even at low temperatures.

Figure 3B shows the ^{1}H NMR spectra of **13**, **14**, and a 1:1 mixture of **13** and **14** at 223 K. From these spectra, no significant change in the spectra was observed. Therefore, it was concluded that an A−C base pair in $CDCl_3$ cannot be formed between **13** and **14**.

Figure 3C shows the ^{1}H NMR spectra of **12** and a 1:1 mixture of **12** and **14** at 223 K. Compared with the ^{1}H NMR spectrum of a 1:1 mixture of **13** and **14**, the amide proton of **12** in Figure 3C(b) was largely shifted (ca. 3.64 ppm) downfield in Figure 3C(a), when **14** was added to **12**. Moreover, the two amino protons of **14** were detected separately at 8.02 and 7.00 ppm in the presence of an equivalent molar amount of **12** at low temperature in Figure 3C(a). It was also found that the 5H proton of **12** did not shift appreciably when **14** was added to **12**. This result implies that the intramolecular hydrogen bonding of the carbonyl oxygen of the methoxycarbonyl group with the 5-vinyl proton remains intact upon formation of the base pair. Therefore, the base pair mode depicted in the form **11b** in Figure 2 was ruled out.

These results suggested that 4-*N*-methoxycarbonyldeoxycytidine derivatives can form a base pair with deoxyadenosine more easily than the deoxycytidine derivatives and the amide proton of 4-*N*-methoxydeoxycytidine plays a major role in forming the mismatched base pair.

To determine the real base pair mode, we synthesized ^{15}N-labeled 4-*N*-methoxycarbonyldeoxycytidine derivatives **15** and **16** by use of a modification of the method for the synthesis of the ^{15}N-labeled ribonucleoside counterparts reported by Ariza,[27] as shown in Scheme 3. Compounds **15** and **16** were obtained from 3′,5′-*O*-bis-(*tert*-butyldimethylsilyl)-2′-deoxyuridine[28] **(17)** in overall yields of 52% and 44%, respectively. Figure 4A shows the ^{1}H NMR spectrum of a 1:1 mixture of the deoxyadenosine derivative **14** and the 4-^{15}N labeled compound **15**, and Figure 4B shows that of a 1:1 mixture of **14** and 3-^{15}N labeled **16**. Only in the case of Figure 4A, split signals having a large coupling constant (90.7 Hz) derived from a covalently bonded ^{15}N and ^{1}H were clearly observed at 11.6 ppm. These results revealed that compound **12** forms a base pair with compound **14** in the amino form in $CDCl_3$, as shown in Figure 4A. These results obtained from the NMR experiments reflect those of T_m experiments above-mentioned.

Conclusion

Incorporation of dC^{ac} into oligodeoxyribonucleotides resulted in stabilization of DNA duplexes, but, in reverse, *N*-acylated deoxycytidine derivatives having a longer hydrophobic side chain destabilized DNA duplexes. To suppress this destabilization, hydrophilic oxygen atoms capable of maintaining the hydration structure in the major groove were introduced into the side chain of the acyl group. Particularly, in the case of dC^{moc}, the modified DNA duplex including five dC^{moc} monomer units at separate positions exhibited a higher T_m value than the natural DNA duplex. The most striking characteristic of 4-*N*-alkoxycarbonyldeoxycytidine derivatives is that they can recognize not only guanosine but also adenosine. NMR and X-ray crystal analysis of DNA duplexes having a C−A mismatched base pair revealed that cytidine could form a Wobble-type base pair with adenosine under acidic conditions by protonation of the N1 position of adenine.[29] In this study, it was concluded that 4-*N*-alkoxycarbonyldeoxycytidine derivatives can form base pairs with not

(27) Ariza, X.; Vilarrasa, J. *J. Org, Chem.* **2000**, *65*, 2827−2829.
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(29) Brown, T.; Kennard, O.; kneale G.; Rabinovich, D. *Nature* **1986**, *320*, 552−555. Patel, D.; Kozlowski, S. A.; Ikuta, S.; Itakura, K. *Biochemistry* **1984**, *23*, 3218−3226.

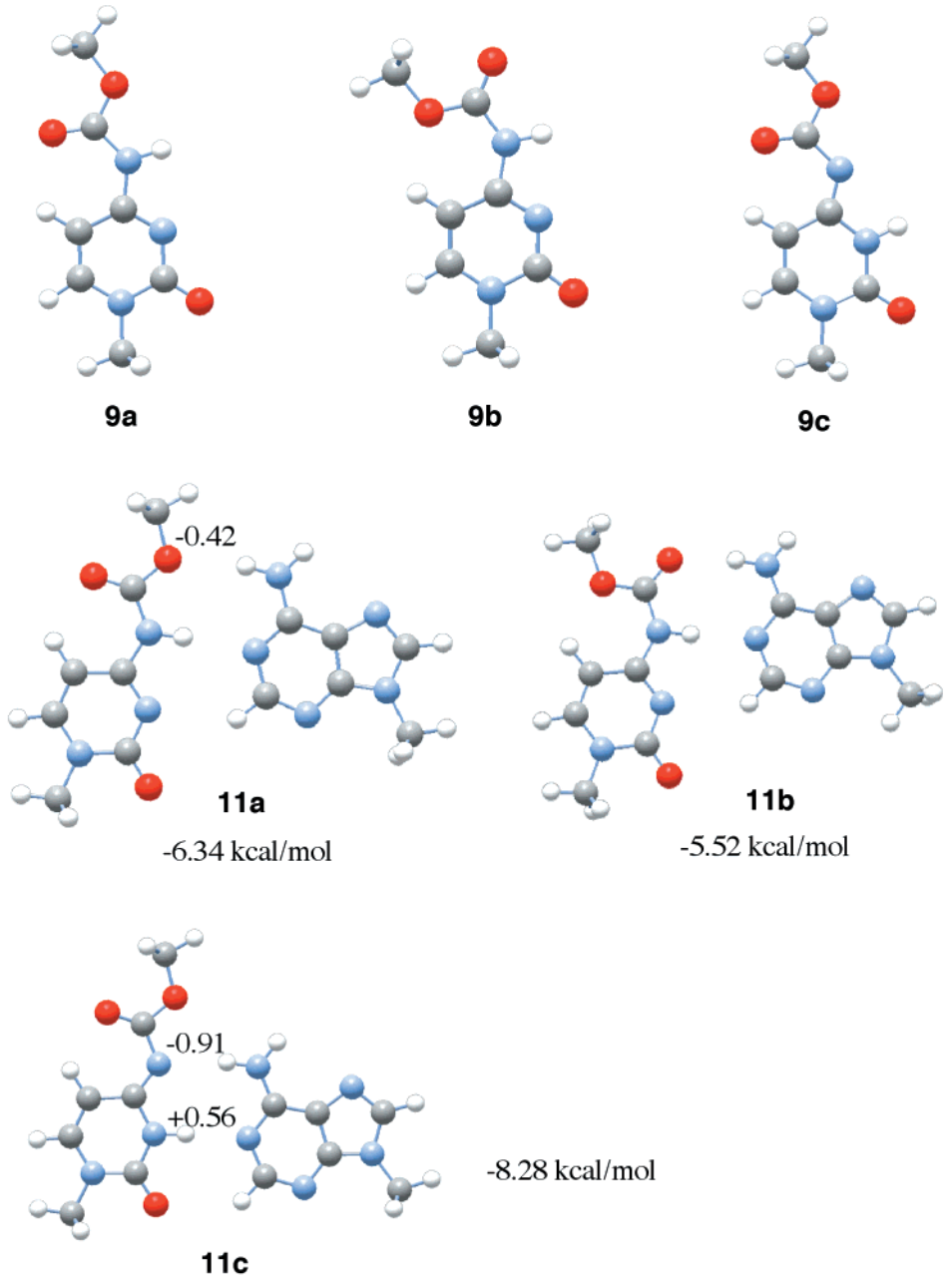


Figure 2. Energy-optimized structures of the conformers **9a**,**b** and tautomer **9c** of 4-*N*-methoxycarbonyl-1-methylcytosine by ab initio MO calculation at the B3LYP/6-31++G** and energy-optimized structure of base pairs between **9a**−**c** and 9-methyladenosine (10) by ab initio MO calculation at the HF/6-31+G**.

Table 3. Relative Difference in Energy (kcal/mol) of the Tautomers 9a−c at the Selected Basis Sets

basis set	9a	9b	9c
HF/6-31G* (kcal/mol) $\epsilon = 0$	0	+3.76	+2.64
Hf/6-31++G** (kcal/mol) $\epsilon = 0$	0	+3.70	+2.82
1-PCM,HF/6-31++G**//HF/6-31++G** (kcal/mol) $\epsilon = 40$	0	+4.77	+6.00
B3LYP/6-31++G** (kcal/mol) $\epsilon = 0$	0	+3.07	+3.01

only deoxyguanosine but also deoxyadenosine under even neutral conditions by using a geometry different from the naturally occurring mismatched base pair of the Wobble type. This inherent property of 4-*N*-alkoxycarbonyldeoxycytidine derivatives would provide new insight in studies associated with DNA mutagenesis.[30] Although 4-*N*-alkoxycarbonylcytidine derivatives have not been discovered from naturally occurring RNAs such as tRNAs and rRNAs, there is a possibility that these modified nucleosides would enable us to artificially change the codon box when incorporated into the anticodon first letter of tRNAs. Further studies are under way in this direction.

Experimental Section

General Remarks. ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded at 270, 68, and 109 MHz, respectively. The chemical shifts were measured from tetramethylsilane or DSS for ^{1}H

(30) Singer, B.; Kusmierek, J. T. *Annu. Rev. Genet.* **1982**, *52*, 655−693.

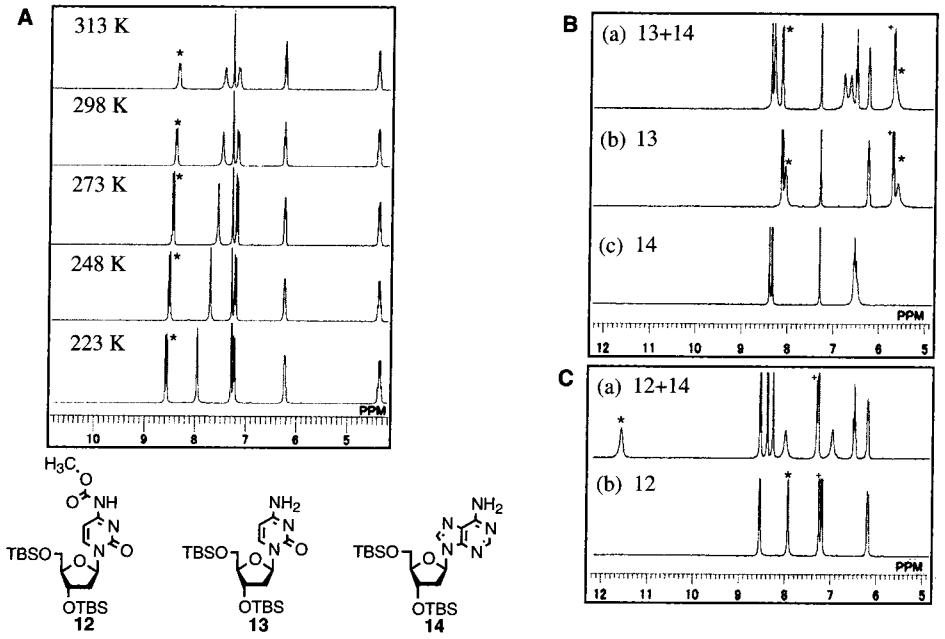
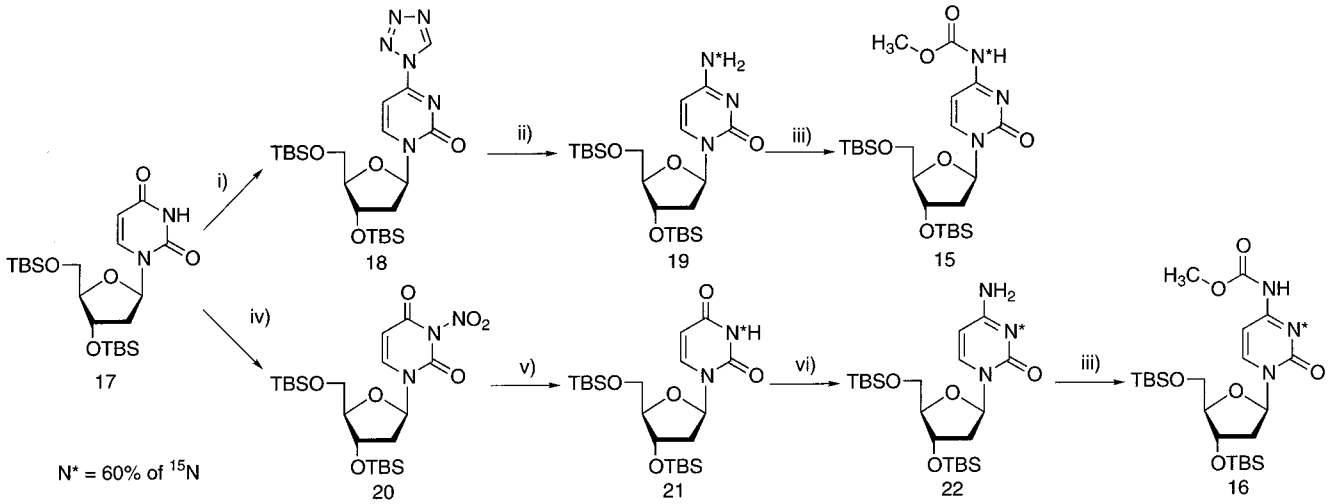


Figure 3. Panel A: ^{1}H NMR spectra of **12** in $CDCl_3$ at various temperatures. The symbol "*" refers to the amide proton of **12**. Panel B: ^{1}H NMR spectra at 223 K ($CDCl_3$) of a mixture of **13** and **14** (a), compound **13** (b), and compound **14** (c). The symbols "*" and "+" in (a) and (b) refer to the amino protons and the 5H proton, respectively, of **13**. Panel C: ^{1}H NMR spectra at 223 K ($CDCl_3$) of a 1:1 mixture of **12** and **14** (a) and compound **12** (b). The symbols "*" and "+" in (a) and (b) refer to the amide proton and the 5H proton, respectively, of dC^{moc} **12**.

Scheme 3[a]



[a] Key: (i) 1.2 equiv of diphenyl phosphate, 2 equiv of 1*H*-tetrazole, 2 equiv of TsCl, pyridine, rt, 2 d; (ii) 1.2 equiv of $^{15}NH_4Cl$, 1.2 equiv of KOH, 1.4 equiv of Et_3N, CH_3CN-H_2O (3:1, v/v), rt, 24 h; (iii) 1.2 equiv of methyl chloroformate, 1.5 equiv of pyridine, CH_2Cl_2, 1 h; (iv) 4 equiv of $(CF_3CO)_2O$, 2 equiv of NH_4NO_3, CH_2Cl_2, 0 °C, 15 min; (v) 1.3 equiv of $^{15}NH_4Cl$, 1.1 equiv of KOH, 1.3 equiv of Et_3N, CH_3CN-H_2O (3:1, v/v), rt, 6 d; (vi) 1.2 equiv of diphenyl phosphate, 2 equiv of 1*H*-tetrazole, 2 equiv of TsCl, pyridine, rt, 2 d, then excess NH_3 aq, rt, 2 h.

NMR spectra, $CDCl_3$ (77 ppm), or DSS (0 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. MALDI TOF mass spectra were taken on a Voyager RP. 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 Gen-Pak FAX column (Waters, 4.6 × 100 mm) on an LC module 1 with a Waters M-741 data module and a Waters column heater with a 10−77% linear gradient of 25 mM phosphate, 1 M sodium chloride buffer (pH 6.0) in 25 mM phosphate buffer (pH 6.0) at 50 °C at a flow rate of 1.0 mL/min for 30 min. Pyridine was distilled two times from *p*-toluenesulfonyl chloride and from calcium hydride and then stored over molecular sieves 4A. Compounds **13**, **14** and **17** were synthesized by the method reported by Ogilvie.[28] Elemental analyses were performed by the Microanalytical Laboratory, Tokyo Institute of Technology at Nagatsuta.

Preparation of Thymidine-Loaded Solid Support via Silanediyl Linker 8. Diisopropylsilanediyl ditriflate (180 μL, 0.6 mmol) and 1,8-diazabicyclo[5.4.0]-7-undecene (269 mg, 1.8 mmol) were dissolved in dry CH_3CN (2 mL). 5′-*O*-(4,4′-Dimethoxytrityl)thymidine (109 mg, 0.2 mmol) in dry CH_3CN (1 mL) was added dropwise to the solution at −40 °C under argon atmosphere. After being stirred for 1 h at room temperature, ArgoPore (500 mg, 0.4 mmol) was added and stirred for an additional 1 h at room temperature. The solid support

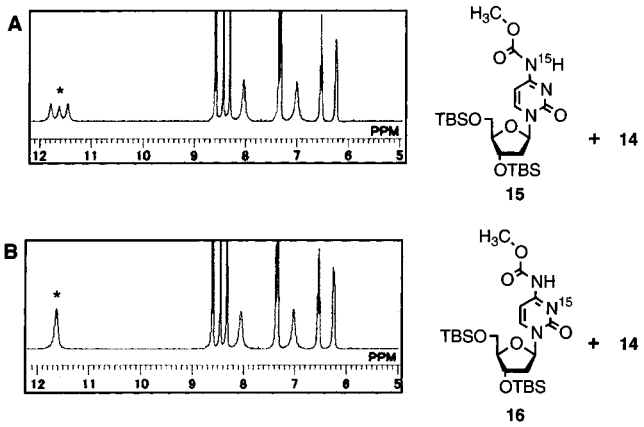


Figure 4. ^{1}H NMR spectra at 223 K ($CDCl_3$) of a 1:1 mixture of 4-*N*-methoxycarbonyl −3′,5′-bisTBDMS deoxycytidine (**15**) and **14** (A) and a 1:1 mixture of 4-*N*-methoxycarbonyl −3′,5′-*O*-bisTBDMS deoxycytidine (**16**) and **14** (B). The symbol "*" refers to the amide proton of **15** and **16**.

was filtered and washed with pyridine. The hydroxyl groups were blocked by treatment with 0.1 M 4-(*N*,*N*-dimethyamino)-pyridine and 10% $(CH_3CO)_2O$ in pyridine. The resulting support **6** was filtered, washed with pyridine, and dried. The loading amount of thymidine was estimated by DMTr cation assay to be 51.6 μmol/g.

Stability of DMT-T-Support toward Tetrabutylammonium Fluoride. The DMT-T-ArgoPore gel support **6** (19.4 mg, 1.0 μmol) was suspended in a solution of tetrabutylammonium fluoride hydrate (131 mg, 0.5 mmol) and acetic acid (30 mL, 0.5 mmol) in tetrahydrofuran (500 μL). After 1 h, the support was filtered and washed with pyridine (1 mL × 3) and CH_2Cl_2 (1 mL × 3). The support was treated with 1% TFA in CH_2Cl_2, and the TFA solution was filtered. The filtrate was concentrated to dryness, and the residue was dissolved in perchloric acid−ethanol (3:2, v/v) to estimate the amount of the released DMTr cation by measurement of the UV absorbance at 498 nm (ϵ = 71 700).

Typical Procedure for Solid-Phase Synthesis. Each cycle of chain elongation consisted of detritylation (1 M zinc bromide in CH_2Cl_2-*i*-PrOH (85/15, v/v), 1 mL, 40 min), washing (pyridine (1 mL × 3), CH_3CN (1 mL × 3)), coupling (0.1 M amidite units, 0.4 M 1*H*-tetrazole in CH_3CN (200 μL), 5 min), and washing (CH_3CN (1 mL × 3), pyridine (1 mL × 3)), oxidation (0.1 M I_2, THF−pyridine−H_2O (10/10/1, v/v/v), 2 min), washing (pyridine (1 mL × 3), CH_3CN (1 mL × 3), CH_2Cl_2 (1 mL × 3)). Generally, the average yield per cycle was estimated to be 97−99% by the DMTr cation assay. After chain elongation, the DMTr group was removed by treatment with 1 M zinc bromide in CH_2Cl_2−*i*-PrOH (85/15, v/v, 1 mL) for 40 min, and the resin was washed with pyridine(1 mL × 3) and CH_2Cl_2(1 mL × 3). The cyanoethyl group was removed by treatment with DBU−CH_3CN (9/1, v/v, 500 mL) for 1 min, and the resin was washed with CH_3CN (1 mL × 3). The oligomer was released from the ArgoPore resin by treatment with tetrabutylammonium fluoride hydrate (131 mg, 0.5 mmol) and acetic acid (30 mL, 0.5 mmol) in tetrahydrofuran (500 mL) for 1 h. The ArgoPore resin was removed by filtration and washed with CH_3CN(1 mL × 3). The filtrate was desalted using gel filtration and purified by anion-exchange HPLC. The oligomers isolated were analyzed by nuclease digestion and MALDI TOF mass spectrometry.

T_m Measurement. An appropriate oligonucleotide and its complementary DNA 13 mer d(A_6GA_6) were dissolved in a buffer consisting of 1.0 M NaCl, 10 mM sodium phosphate, and 0.1 mM EDTA adjusted to pH 7.0. The solution containing oligonucleotides was kept at 60 °C for 10 min for complete dissociation of the duplex to single strands, cooled at the rate of −1.0 °C/min, and kept at 0 °C for 10 min. After that, the melting temperatures (T_m) were determined at 260 nm using a Beckman DU 650 spectrophotometer by increasing the temperature at the rate of 1.0 °C/min.

2′-Deoxy-4-*N*-methoxycarbonyl-5′-*O*-(4,4′-dimethoxytrityl)cytidine (3a). General Procedure. 2′-Deoxycytidine hydrochloride (**1**) (2.64 g, 10 mmol) and hexamethyldisilazane (1.1 mL, 50 mmol) was dissolved in CH_3CN (100 mL), and the mixture was stirred for 30 min at 60 °C. After evaporation, the residue and pyridine (1.2 mL, 15 mmol) were redissolved in CH_2Cl_2 (100 mL). Methyl chloroformate (1.2 mL, 15 mmol) was added dropwise over 10 min to the solution at 0 °C. After the mixture was stirred for 30 min, the organic layer was washed three times with 5% $NaHCO_3$ (aq), dried over $MgSO_4$, filtered, and evaporated under reduced pressure. Pyridine−concentrated NH_3 (1:1, v/v, 100 mL) was added to the residue. After being stirred for another 30 min, the solution was evaporated to dryness. The residue was diluted with $CHCl_3$ (100 mL) and washed three times with 5% $NaHCO_3$ (aq). The organic layer was combined, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was dried by repeated coevaporation with dry pyridine and finally dissolved in dry pyridine (100 mL). To the solution was added 4,4′-dimethoxytrityl chloride (4.07 g, 12 mmol), and the mixture was stirred at room temperature for 3 h. The mixture was concentrated to half volume, diluted with $CHCl_3$, and washed three times with 5% $NaHCO_3$ (aq), and the aqueous layer was back-extracted with $CHCl_3$. The organic layer and the washings were combined, dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was chromatographed on a column of silica gel (100 g) with $CHCl_3$ containing 1% pyridine, applying a gradient of methanol (1−2%) to give **3a** (5.41 g, 92%) as colorless foam: ^{1}H NMR ($CDCl_3$) δ 2.22 (1H, m), 2.75 (1H, m), 3.34 (1H, br), 3.45 (2H, dd, J = 3.0, 10.7 Hz), 3.79 ÅA3.74 (9H, 2s), 4.15 (1H, m), 4.50 (1H, m), 6.27 (1H, dd, J = 5.4, 5.6 Hz), 6.84 (4H, d, J = 8.4 Hz), 6.99 (1H, d, J = 7.4 Hz), 7.05−7.40 (9H, m), 7.83 (1H, br), 8.22 (1H, d, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$) δ 41.7, 52.6, 54.8, 62.6, 70.4, 86.2, 86.3, 87.0, 94.7, 112.8, 126.6, 127.5, 127.7, 129.6, 129.6, 134.9, 135.1, 143.8, 158.1, 158.1, 144.0, 152.7, 154.9, 162.2. Anal. Calcd for $C_{32}H_{33}N_3O_8\cdot{}^1/_2H_2O$: C, 64.42; H, 5.74; N, 7.04. Found: C, 64.19; H, 5.52; N, 6.89.

2′-Deoxy-4-*N*-butoxycarbonyl-5′-*O*-(4,4′-dimethoxytrityl)cytidine (3b). With use of the general procedure, compound **3b** was obtained from 2′-deoxycytidine hydrochloride (**1**) (527 mg, 2 mmol) as a colorless foam (1.08 g, 86%): ^{1}H NMR ($CDCl_3$) δ 0.94 (3H, t, J = 7.3 Hz), 1.39 (2H, m), 1.63 (2H, m), 2.23 (1H, m), 2.72 (1H, m), 3.22 (1H, br), 3.45 (2H, m), 3.80 (6H, s), 4.15−4.23 (3H, m), 5.00 (1H, m), 6.27 (1H, dd, J = 5.6, 5.6 Hz), 6.84 (4H, d, J = 8.9 Hz), 7.00 (1H, d, J = 7.3 Hz), 7.23−7.41 (9H, m), 7.61 (1H, br), 8.22 (1H, d, J = 7.3 Hz); ^{13}C NMR ($CDCl_3$) δ 13.7, 19.0, 30.6, 66.0, 55.2, 42.1, 62.7, 70.8, 87.2, 88.4, 86.7, 94.7, 113.1, 126.9, 127.8, 129.8, 129.9, 135.2, 135.4, 144.0, 144.1, 144.1, 152.3, 155.2, 158.4, 162.1. Anal. Calcd for $C_{35}H_{39}N_3O_8\cdot H_2O$: C, 64.90; H, 6.38; N, 6.49. Found: C, 65.11; H, 6.02; N, 6.48.

2′-Deoxy-4-*N*-methoxyethoxycarbonyl-5′-*O*-(4,4′-dimethoxytrityl)cytidine (3c). With use of the general procedure, compound **3c** was obtained from 2′-deoxycytidine hydrochloride (**1**) (527 mg, 2 mmol) as a colorless foam (1.07 g, 85%): ^{1}H NMR ($CDCl_3$) δ 2.26 (1H, m), 2.51 (1H, br), 2.55 (1H, m), 3.40 (3H, s), 3.46 (2H, m), 3.63 (2H, t, J = 4.6 Hz), 3.80 (6H, s), 4.11 (1H, m), 4.33 (2H, t, J = 4.6 Hz), 4.49 (1H, m), 6.25 (1H, dd, J = 5.6, 5.9 Hz), 6.85 (4H, d, J = 8.3 Hz), 6.99 (1H, d, J = 7.3 Hz), 7.16−7.41 (9H, m), 8.23 (1H, d, J = 7.3 Hz); ^{13}C NMR ($CDCl_3$) δ 42.0, 55.1, 58.8, 62.7, 64.8, 70.1, 70.7, 86.4, 86.7, 87.2, 94.8, 113.1, 126.8, 127.7, 127.9, 129.8, 129.8, 135.2, 135.3, 144.0, 144.1, 152.2, 155.1, 162.1. Anal. Calcd for $C_{34}H_{37}N_3O_9\cdot{}^1/_2H_2O$: C, 63.74; H, 5.98; N, 6.56. Found: C, 63.80; H, 5.97; N, 6.30.

2′-Deoxy-4-*N*-methoxycarbonyl-5′-*O*-(4,4′-dimethoxytrityl)cytidine 3′-(2-Cyanoethyl *N*,*N*-diisopropylphosphoramidite) (4a). Compound **3a** (580 mg, 1.0 mmol) was rendered anhydrous by successive coevaporations with dry pyridine (1 mL × 3) and dissolved in dry CH_2Cl_2 (10 mL). To the mixture were added diisopropylethylamine (313 μL, 1.8 mmol) and chloro(2-cyanoethoxy)(*N*,*N*-diisopropylamino)phosphine (335

μL, 1.5 mmol). The resulting mixture was stirred for 1 h and extracted with 5% $NaHCO_3$ and water. The usual workup followed by silica gel column chromatography eluted with hexanes−ethyl acetate containing 0.5% triethylamine gave **4a** (701 mg, 89%): ^{1}H NMR ($CDCl_3$) δ 1.05−1.29 (12H, m), 2.28 (1H, m), 2.43 (1H, t, J = 6.4 Hz), 2.61 (1H, t, J = 6.4 Hz), 2.74 (1H, m), 3.60−3.80 (15H, m), 4.19 (1H, m), 4.61(1H, m), 6.25 (1H, dd, J = 5.6 Hz, J = 4.9 Hz), 6.82−6.97 (5H, m), 7.22−7.42 (9H, m), 8.19, 8.28 (1H, d, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$) δ 20.1, 20.2, 20.3, 20.4, 21.5, 24.6, 24.6, 40.8, 41.1, 43.0, 43.1, 43.2, 43.3, 52.9, 55.2, 55.1, 58.0, 58.3, 61.8, 62.3, 71.3, 72.3, 85.4, 85.5, 85.5, 86.7, 94.5, 113.1, 117.2, 117.3, 126.9, 127.8, 127.9, 128.0, 128.0, 129.8, 129.9, 130.0, 135.0, 135.1, 135.2, 143.8, 144.0, 152.7, 154.7, 158.4, 162.0; ^{31}P NMR ($CDCl_3$) δ 149.4, 149.9. Anal. Calcd for $C_{41}H_{50}N_5O_9P\cdot{}^1/_2H_2O$: C, 61.80; H, 6.45; N, 8.79. Found: C, 61.76; H, 6.19; N, 8.72.

4-*N*-Butoxycarbonyl-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)cytidine 3′-(2-Cyanoethyl *N*,*N*-diisopropylphosphoramidite) (4b). With use of the general procedure, compound (**4b**) was obtained from 4-*N*-butoxycarbonyl-5′-*O*-(4,4′-dimethoxytrityl)deoxycytidine (**3b**) (378 mg, 0.6 mmol) as colorless foam (414 mg, 83%): ^{1}H NMR ($CDCl_3$) δ 0.95 (3H, t, J = 7.26), 1.06−1.44 (14H, m), 1.64 (2H, m), 2.36 (1H, m), 2.44, 2.61 (2H, t, J = 6.3 Hz), 2.74 (1H, m), 3.38−3.82 (15H, m), 4.15−4.21 (3H, m), 4.62 (1H, m), 6.45 (1H, m), 6.82−6.90 (4H, m), 6.93 (1H, dd, J = 7.3, 7.6 Hz), 7.23−7.42 (9H, m), 8.18, 8.28 (1H, d, J = 7.3, 7.6 Hz); ^{13}C NMR ($CDCl_3$) δ 13.5, 19.1, 20.1, 20.2, 20.3, 20.3, 24.4, 24.6, 24.6, 30.5, 41.5, 41.6, 43.0, 43.1, 43.3, 43.3, 55.3, 55.3, 55.3, 58.1, 58.3, 61.9, 62.3, 66.0, 71.7, 72.3, 85.5, 85.6, 85.7, 87.0, 94.5, 113.2, 117.2, 117.4, 127.4, 127.8, 127.9, 128.0, 128.0, 130.1, 130.2, 130.2, 135.4, 135.4, 144.1, 144.2, 152.3, 155.1, 158.6, 162.0; ^{31}P NMR ($CDCl_3$) δ 149.3, 149.9. Anal. Calcd for $C_{44}H_{56}N_5O_9P$: C, 63.68; H, 6.80; N, 8.44. Found: C, 63.81; H, 6.77; N, 8.21.

2′-Deoxy-4-*N*-methoxyethoxycarbonyl-5′-*O*-(4,4′-dimethoxytrityl)cytidine 3′-(2-Cyanoethyl *N*,*N*-diisopropylphosphoramidite) (4c). With use of the general procedure, compound (**4c**) was obtained from 2′-deoxy-4-*N*-methoxyethoxycarbonyl-5′-*O*-(4,4′-dimethoxytrityl) cytidine (**3c**) (190 mg, 0.3 mmol) as colorless foam (130 mg, 52%): ^{1}H NMR ($CDCl_3$) δ 1.06−1.29 (12H, m), 2.29 (1H, m), 2.44, 2.62 (2H, t, J = 6.4 Hz), 2.74 (1H, m), 3.36−3.83 (11H, m), 3.81 (3H, s), 3.83 (3H, 1s), 4.20 (1H, m), 4.33 (2H, m), 4.61 (1H, m), 6.25 (1H, m), 6.84 (4H, m), 6.90, 6.93(1H, d, J = 7.2, 7.6 Hz), 7.22−7.41 (9H, m), 8.19, 8.29 (1H, d, J = 7.2, 7.6 Hz); ^{13}C NMR ($CDCl_3$) δ 20.1, 20.2, 20.2, 20.3, 24.5, 24.7, 24.7, 41.6, 41.7, 43.0, 43.2, 43.3, 43.3, 55.2, 55.2, 58.0, 58.2, 58.9, 61.9, 62.2, 64.6, 70.3, 71.6, 72.2, 85.5, 85.6, 85.6, 87.1, 94.6, 113.2, 117.3, 117.4, 127.3, 127.8, 127.9, 128.1, 128.1, 130.1, 130.2, 130.2, 135.3, 135.4, 135.4, 144.0, 144.2, 152.2, 155.0, 158.5, 162.0; ^{31}P NMR ($CDCl_3$) δ 149.4, 150.0. Anal. Calcd for $C_{43}H_{54}N_5O_9P$: C, 62.08; H, 6.54; N, 8.42. Found: C, 62.15; H, 6.52; N, 8.27.

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-2′-deoxy-4-*N*-methoxycarbonylcytidine (12). 3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)deoxycytidine[28] (3.7 g, 8 mmol) and pyridine (1.0 mL, 12 mmol) were dissolved in CH_2Cl_2 (80 mL). Methyl chloroformate (740 mL, 9.6 mmol) was added dropwise over 10 min to the solution at 0 °C, and the mixture was stirred at room temperature for 1 h. The mixture was concentrated to half volume, diluted with $CHCl_3$, and washed three times with 5% $NaHCO_3$ (aq). The aqueous layer was back-extracted with $CHCl_3$. The organic layer and the washings were combined and dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was chromatographed on a column of silica gel (80 g) with $CHCl_3$, applying a gradient of methanol (1−2%) to give **12** (3.3 g, 80%) as colorless foam: ^{1}H NMR ($CDCl_3$) δ 0.11 (6H, s), 0.12 (6H, s), 0.88 (9H, s), 0.93 (9H, s), 2.10−2.18 (1H, m), 2.47−2.57 (1H, m), 3.76−3.98 (6H, m), 4.35−4.41 (1H, m), 6.24 (1H, dd, J = 5.4 Hz, J = 5.4 Hz), 7.16 (1H, d, J = 7.3 Hz), 7.46 (1H, br), 8.38 (1H, d, J = 7.3 Hz); ^{13}C NMR ($CDCl_3$) δ −5.6, −5.5, −5.0, −4.6, 17.9, 18.3, 25.7, 25.9, 42.3, 53.0, 61.7, 70.0, 86.7, 87.7, 94.2, 144.5, 152.9, 154.9, 162.0; MS m/z calcd for $C_{23}H_{44}N_3O_6Si_2^+$ 514.2769, found 514.2781.

1-[3,5-*O*-Bis(*tert*-butyldimethylsilyl)-2-deoxy-β-D-ribofuranosyl]-4-(1*H*-tetrazol-1-yl)pyrimidin-2(1*H*)-one (18). Compound **17** (500 mg, 1.1 mmol), 1*H*-tetrazole (154 mg, 2.2 mmol), diphenyl phosphate (330 mg, 1.3 mmol), and tosyl chloride (419 mg, 2.2 mmol) were dissolved in pyridine (3 mL), and the mixture was stirred at room temperature for 2 days. Afterward, 1 mL of water was added, and the solution was poured into aqueous Na_2CO_3 (saturated) and extracted with CH_2Cl_2. The solvent was dried (Na_2SO_4) and removed by coevaporation with toluene. The residue was purified by column chromatography (hexanes−ethyl ether 60:40) to afford **18** (436 mg, 78%): ^{1}H NMR ($CDCl_3$) δ 0.07−0.16 (12H, m), 0.89−0.95 (18H, m), 2.17−2.26 (1H, m), 2.59−2.69 (1H, m), 3.82 (1H, dd, J = 2.5 Hz, J = 12.0 Hz), 4.01−4.06 (2H, m), 4.40 (1H, m), 6.25 (1H, dd, J = 4.3 Hz, J = 6.6 Hz), 7.12 (1H, d, J = 7.2 Hz), 8.87 (1H, d, J = 7.2 Hz), 9.60 (1H, s); ^{13}C NMR ($CDCl_3$) δ −5.4, −4.9, −4.5, 17.9, 18.3, 25.6, 25.9, 42.2, 61.5, 69.6, 87.8, 88.1, 94.1, 140.4, 148.1, 153.6, 156.8. MS m/z calcd for $C_{22}H_{41}N_6O_4Si_2^+$ 509.2728, found 509.2738.

[4-^{15}NH$_2$]-3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-2′-deoxycytidine (19). $^{15}NH_4Cl$ (144 mg, 2.6 mmol) and KOH (85%, 174 mg, 2.6 mmol) were placed in a 30-ml round flask and sealed with a septum. Water (4.4 mL), CH_3CN (4.4 mL), Et_3N (430 mL, 3.1 mmol), and a solution of 1-[3,5-*O*-Bis(*tert*-butyldimethylsilyl)-2-deoxy-β-D-ribofuranosyl]-4-(1*H*-tetrazol-1-yl)pyrimidin-2(1*H*)-one **(18)** (1.1 g, 2.2 mmol) in CH_3CN (8.8 mL) were then added sequentially with syringe equipment, avoiding leakage of ammonia. After the mixture was stirred for 24 h, the solvent was removed, and the residue was diluted with $CHCl_3$ and washed three times with 5% $NaHCO_3$ (aq). The organic layer was dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was chromatographed on a column of silica gel (20 g) with $CHCl_3$, applying a gradient of methanol (1−2%) to give **19** (964 mg, 80%) as colorless foam: ^{1}H NMR ($CDCl_3$) δ 0.05 (6H, s), 0.11 (6H, s), 0.88−0.93 (18H, m), 2.08−2.13 (1H, m), 2.38−2.45 (1H, m), 3.74−3.94 (3H, m), 4.37 (1H, m), 5.62 (1H, d, J = 7.4 Hz), 6.26 (1H, dd, J = 5.7 Hz), 8.01 (1H, d, J = 7.3 Hz); ^{13}C NMR ($CDCl_3$) δ −5.7, −5.6, −5.0, −4.7, 17.7, 18.1, 25.6, 25.7, 41.8, 61.8, 70.3, 85.3, 86.8, 94.7, 139.9, 155.7, 165.7 (d, J = 20.1 Hz); MS m/z calcd for $C_{23}H_{42}N_2{}^{15}NO_4Si_2^+$ 457.2684, found 457.2685.

[4-^{15}NH$_2$]-3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-2′-deoxy-4-*N*-methoxycarbonylcytidine (15). Compound **15** was obtained from [4-$^{15}NH_2$]-3′,5′-*O*-bis(*tert*-butyldimethylsilyl)-2′-deoxycytidine **(19)** (183 mg, 0.4 mmol) as colorless foam (170 mg, 83%) with use of the same procedure of compound **12**: ^{1}H NMR ($CDCl_3$) δ 0.06 (6H, s), 0.12 (6H, s), 0.88 (9H, s), 0.93 (9H, s), 2.11−2.16 (1H, m), 2.50−2.55 (1H, m), 3.77−3.98 (6H, m), 4.38−4.40 (1H, m), 6.24 (1H, dd, J = 5.4 Hz, J = 5.4 Hz), 7.17 (1H, d, J = 7.3 Hz), 7.40 (1H, br), 8.38 (1H, d, J = 7.3 Hz); ^{13}C NMR ($CDCl_3$) δ −5.6, −5.5, −5.0, −4.6, 17.8, 18.2, 25.6, 25.8, 42.1, 52.8, 61.8, 69.8, 86.5, 87.5, 94.2, 144.0, 152.9 (d, J = 26.2 Hz), 154.5 (br), 162.1 (d, J = 20.1 Hz); MS m/z calcd for $C_{23}H_{44}N_2{}^{15}NO_6Si_2$ 515.2739, found 515.2751.

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-2′-deoxy-3-nitrouridine (20). Trifluoroacetic anhydride (2.82 mL, 20 mmol) was added to a suspension of finely powdered NH_4NO_3 (800 mg, 10 mmol) in anhydrous CH_2Cl_2 (5 mL), at 0 °C. The mixture was vigorously stirred at room temperature until the solid was dissolved (ca. 1 h) and then cooled again. After addition of 3′,5′-*O*-bis(*tert*-butyldimethylsilyl)-2′-deoxyuridine **(17)** (2.28 g, 5.0 mmol), the mixture was stirred at 0 °C for 15 min, washed with cold phosphate buffer, dried, and evaporated in vacuo. The residue was chromatographed on a column of silica gel (40 g) with hexane, applying a gradient of ethyl acetate (3−5%) to give **20** (2.1 g, 83%) as colorless syrup: ^{1}H NMR ($CDCl_3$) δ 0.09−0.12 (12H, m), 0.90−0.93 (18H, m), 2.10−2.17 (1H, m), 2.34−2.42 (1H, m), 3.76−3.98 (3H, m), 4.43 (1H, m), 5.79 (1H, d, J = 8.2 Hz), 6.25 (1H, dd, J = 5.9 Hz), 8.01 (1H, d, J = 8.2 Hz); ^{13}C NMR ($CDCl_3$) δ −5.5, −5.5, −4.8, −4.7, 18.0, 18.4, 25.7, 25.9, 42.0, 62.3, 71.1, 86.4, 88.2, 100.8, 139.7, 145.2, 155.1; MS m/z calcd for $C_{21}H_{40}N_3O_7Si_2^+$ 502.2405, found 502.2422.

[3-^{15}N]-3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-2′-deoxyuridine (21). $^{15}NH_4Cl$ (357 mg, 6.5 mmol) and KOH (85%, 309 mg, 5.5 mmol) were placed in a 10 mL round-bottom flask and sealed with a septum. Water (12 mL), CH_3CN (12 mL), Et_3N (909 mL, 6.5 mmol), and a solution of 3′,5′-*O*-bis(*tert*-butyldimethylsilyl)-2′-deoxy-3-nitrouridine **(20)** (2.5 g, 5.0 mmol) in CH_3CN (24 mL) were added sequentially, avoiding leakage of ammonia. After the mixture was stirred for 6 days, the solvent was removed, and the residue was diluted with $CHCl_3$ and washed three times with 5% $NaHCO_3$ (aq). The organic layer was dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was chromatographed on a column of silica gel (50 g) with $CHCl_3$, applying a gradient of methanol (1−2%) to give **21** (2.0 g, 87%) as a colorless foam: ^{1}H NMR ($CDCl_3$) δ 0.08−0.11 (12H, m), 0.89−0.92 (18H, m), 2.01−2.11 (1H, m), 2.28−2.37 (1H, m), 3.73−3.94 (3H, m), 4.41−4.43 (1H, m), 5.69 (1H, d, J = 7.9 Hz), 6.29 (1H, dd, J = 5.9 Hz), 7.89 (1H, d, J = 7.9 Hz), 8.43 (1H, d, J = 91.0 Hz); ^{13}C NMR ($CDCl_3$) δ −5.6, −5.5, −4.9, −4.6, 17.9, 18.3, 25.7, 25.8, 41.7, 62.2, 70.9, 85.0, 87.5, 102.0, 139.8, 150.3 (br), 163.6 (br); MS *m*/*z* calcd for $C_{21}H_{41}N^{15}NO_5Si_2^+$ 458.2524, found 458.2537.

[3-^{15}N]-3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-2′-deoxycytidine (22). Compound **21** (500 mg, 1.1 mmol), tetrazole (154 mg, 2.2 mmol), diphenyl phosphate (330 mg, 1.32 mmol), and tosyl chloride (419 mg, 2.2 mmol) were dissolved in pyridine and stirred at room temperature for 2 days. Afterward, concd NH_3 (2 mL) was added to the mixture. After being stirred for 2 h, the mixture was concentrated to half volume, diluted with $CHCl_3$, and washed three times with 5% $NaHCO_3$ (aq). The aqueous layer was back-extracted with $CHCl_3$. The organic layer and the washings were combined and dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was chromatographed on a column of silica gel (80 g) with $CHCl_3$, applying a gradient of methanol (1−2%) to give **22** (436 mg, 87%) as colorless foam: ^{1}H NMR ($CDCl_3$) δ 0.05 (6H, s), 0.10 (6H, s), 0.88−0.92 (18H, m), 2.04−2.13 (1H, m), 2.38−2.47 (1H, m), 3.74−3.95 (3H, m), 4.33−4.40 (1H, m), 5.63 (1H, d, J = 7.4 Hz), 6.26 (1H, dd, J = 5.7 Hz), 8.01 (1H, d, J = 7.3 Hz); ^{13}C NMR ($CDCl_3$) δ −5.7, −5.7, −5.0, −4.7, 17.8, 18.2, 25.6, 25.7, 41.8, 61.7, 70.3, 85.3, 86.9, 94.7, 140.0, 155.7 (d, J = 7.8 Hz), 165.6 (d, J = 6.1 Hz); MS *m*/*z* calcd for $C_{23}H_{44}N_3O_6Si_2^+$ 457.2684, found 457.2685.

[3-^{15}N]-3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-2′-deoxy-4-*N*-methoxycarbonylcytidine (16). Compound **16** was obtained from [3-^{15}N]-3′,5′-*O*-bis(*tert*-butyldimethylsilyl)-2′-deoxycytidine **(22)** (183 mg, 0.4 mmol) as a colorless foam (130 mg, 78%) by use of the same procedure as for compound **12**: ^{1}H NMR ($CDCl_3$) δ 0.06 (6H, s), 0.12 (6H, s), 0.88 (9H, s), 0.93 (9H, s), 2.11−2.17 (1H, m), 2.50−2.55 (1H, m), 3.76−3.98 (6H, m), 4.38−4.40 (1H, m), 6.24 (1H, dd, J = 5.4 Hz, J = 5.4 Hz), 7.16 (1H, d, J = 7.3 Hz), 7.46 (1H, br), 8.38 (1H, d, J = 7.3 Hz); ^{13}C NMR ($CDCl_3$) δ −5.5, −5.4, −5.0, −5.0, −4.6, 17.9, 18.3, 25.7, 25.9, 42.2, 52.9, 61.7, 69.9, 86.6, 87.6, 94.2, 144.1, 152.9, 154.7 (br), 162.0 (br); MS *m*/*z* calcd for $C_{23}H_{44}N_2{}^{15}NO_6Si_2^+$ 5115.2739, found 515.2757.

Enzymatic Treatment of Oligodeoxynucleotides (7a−c and 8) with Snake Venom Phosphodiesterase and Alkaline Phosphatase. Oligodeoxynucleotides (**7a−c** and **8**) (0.5 A_{260}) were each dissolved in 0.05 M Tris-HCl (pH 8.0, 200 mL) containing 0.01 M $MgCl_2$, and 10 mL of SVPD (10 unit, 1.0 unit/mL) in glycerin−water (1:1, v/v) was added. The resulting mixture was incubated at 37 °C for 4 h and heated at 90 °C for 2 min. To the resulting mixture was added 20 μL of alkaline phosphatase (20 unit, 1.0 unit/μL). After being incubated for 3 h, the mixture was heated at 90 °C for 2 min. The mixture was analyzed by reversed-phase HPLC.

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