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Conformational Studies of 4-*N*-Carbamoyldeoxycytidine Derivatives and Synthesis and Hybridization Properties of Oligodeoxyribonucleotides Incorporating these Modified Bases

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Deoxycytidine derivatives modified with various *N*-substituted carbamoyl groups at the 4-amino group were synthesized. The detailed ^{1}H NMR studies suggest that the 3′,5′-*O*-disilylated *N*-carbamoyldeoxycytidine derivative **11** exists as a species having an intramolecular hydrogen bond between the N^3 atom and the carbonyl oxygen atom in MeOD but upon addition of $CDCl_3$, the amount of a homodimer species, having intermolecular hydrogen bonds, gradually increases. Oligodeoxyribonucleotides incorporating various 4-*N*-carbamoyldeoxycytidine derivatives were also synthesized. These modified oligodeoxynucleotides can hybridize with the complementary strands without disturbing the structure of DNA duplexes, hence changing the orientation of the carbamoyl group in such a manner that a stable Watson–Crick base pair can be formed with the guanine base at the opposite site. It should be noted that the base recognition ability (G against T, C and A) of these modified cytosine bases can be preserved satisfactorily. These results suggest that the 4-*N*-carbamoyl group is useful as a backbone structure of the linker between various functional residues and oligonucleotides.

Introduction

We have recently reported the hybridization properties of oligodeoxynucleotides containing 4-*N*-acyldeoxycytidine,[1,2] 4-*N*-alkoxycarbonyldeoxycytidine[3] and 4-*N*-(*N*-arylcarbamoyl)deoxycytidine derivatives,[4] as shown in Figure 1.

Despite the modification of the 4-amino group that is involved in the canonical hydrogen bonding, our results showed that acylation or alkoxycarbonylation of the 4-amino group of deoxycytidine not only allowed formation of the Watson–Crick base pair[1–3] but also increased the hybridization affinity for the complementary guanine base.[1,2] The ^{1}H NMR analysis and the ab initio MO calculations revealed that these 4-*N* substituents were fixed in the same "*proximal*" geometry as that reported in the case of 4-*N*-acetylcytidine found in tRNA[5–11] and rRNA.[12,13] From the crystal[14] and NMR[15] structures reported to date, it was concluded that the carbonyl oxygen atom of these acyl-type groups is orientated in close proximity to 5-C. For example, the acetyl group is geometrically fixed, so that it allows formation of the Watson–Crick base pair

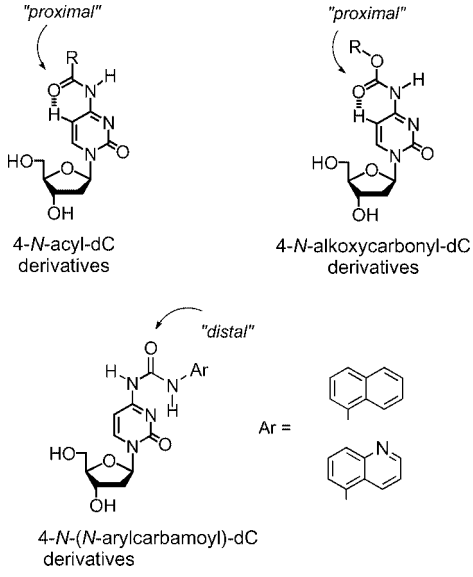

Figure 1. Structures for 4-*N*-acydeoxycytidine, 4-*N*-alkoxycarbonydeoxycytidine and 4-*N*-(*N*-arylcarbamoyl)deoxycytidine derivatives.

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owing to an intramolecular hydrogen bond between the carbonyl oxygen atom and the 5-vinyl proton of the cytosine ring.

The *exo*-amino groups of the cytosine and adenine moieties in oligodeoxyribonucleotides have been used as convenient sites to introduce fluorescence groups[16,17] or to attempt to acquire the triplex-forming ability[18] through carbamoyl-type linkers. However, the base-pairing ability as well as the base-recognition ability of these modified bases having the *N*-carbamoyl structures has never been examined to date. Several previous studies suggested that 4-*N*-(*N*-alkylcarbamoyl)deoxycytidine derivatives **1a**–**e** could form two types of intramolecular hydrogen bonds, as shown in Figure 2.[19]

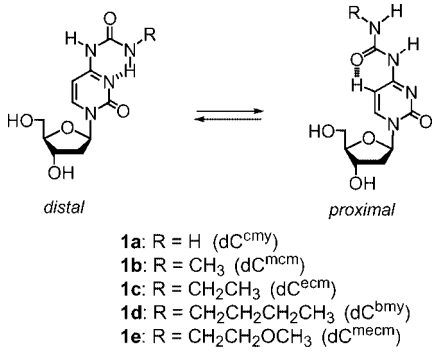


Figure 2. Intramolecular hydrogen bonding patterns of 4-*N*-carbamoyldeoxycytidine derivatives **1a**–**e**.

Kumar et al. reported the crystal structures of 4-*N*-(ureidocarbonyl)deoxycytidine (**2**) and 4-*N*-[*N*-(methoxycarbonyl)carbamoyl]-3′,5′-di-*O*-acetyldeoxycytidine (**3**).[19] They found that in the former the imido proton of the ureidocarbonyl [$H_2NC(O)NHC(O)$–] group forms an intramolecular hydrogen bond with the cytosine ring nitrogen atom so that its orientation becomes "*distal*" to 5-C of the pyrimidine ring, as shown in Figure 3. The latter exists as a homodimer with four intermolecular hydrogen bonds at the Watson–Crick base-pairing site. This structure has a conformation "*proximal*" to 5-C, which allows formation of an intramolecular hydrogen bond between the carbonyl oxygen atom of the carbamoyl group and 5-H of the pyrimidine ring in a manner similar to that of 4-*N*-aceyldeoxycytidine[14] or 4-*N*-alkoxycarbonyldeoxycytidine.[3]

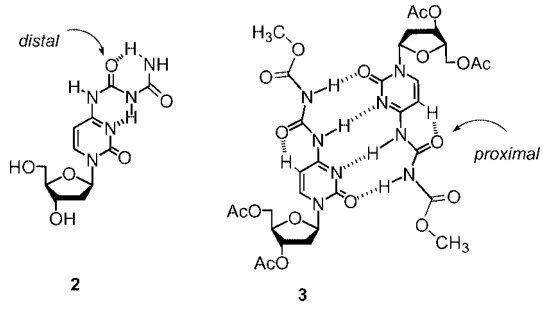


Figure 3. Crystal structures of of 4-*N*-(ureidocarbonyl)-dC (**2**) and 4-*N*-[*N*-(methoxycarbonyl)carbamoyl]-3′,5′-di-*O*-acetyl-dC (**3**).

These early papers suggested that the orientation of the 4-*N*-carbamoyl group is flexible depending on the nitrogen substituents and thereby the 4-*N*-carbamoylcytosine base in oligonucleotides can form a Watson–Crick base pair with guanine under certain conditions which allow the "*proximal*" conformation. However, no papers have been reported about the conformational behavior of 4-*N*-(*N*-alkylcarbamoyl)deoxycytidine derivatives when they are incorporated into oligodeoxyribonucleotides.

In this paper, we report the detailed conformational studies of 4-*N*-(*N*-alkylcarbamoyl)deoxycytidines **1a**–**e** and the hybridization and base-recognition properties of oligodeoxyribonucleotides containing **1a**–**e**.

Results and Discussion

Conformational Studies of 1-Methyl-4-*N*-carbamoylcytosine by Use of Ab Initio MO Calculations in Vacuo

For the comprehensive conformational analysis of the *N*-unsubstituted carbamoyl group of 4-*N*-carbamoyldeoxycytidine (**1a**: dC^{cmy}) the relative energies of the possible conformational isomers **4a**–**f** (see Figure 4) of 4-*N*-carbamoyl-1-methylcytosine were calculated by using the Gaussian 98 package program.[20]

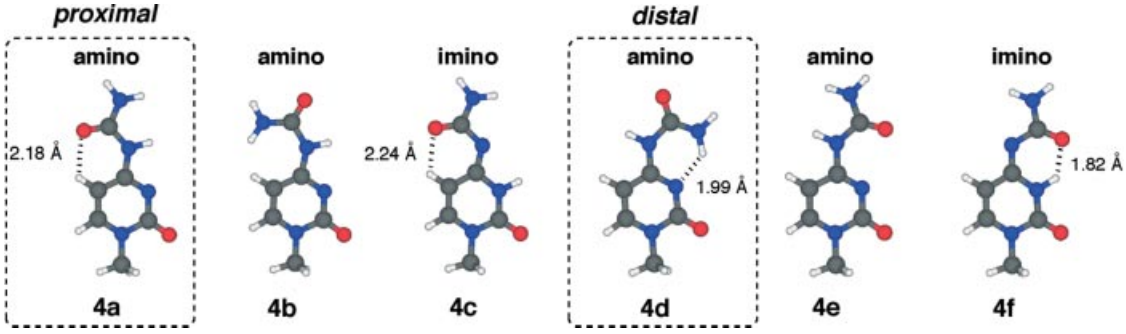


Figure 4. Energy-optimized structures of the geometric and tautomeric isomers **4a**–**f** of 4-*N*-carbamoyl-1-methylcytosine by DFT calculations at the B3LYP/6-31++G** level. The orientation of the 4-*N*-substituents shown in **4a**, **4b** and **4c** are defined as "proximal" and that of **4d**, **4e** and **4f** as "distal". The intramolecular hydrogen bond is indicated by a dotted line with the distance between the hydrogen bonding atoms.

Table 1. Relative difference in energy [kcal/mol] between **4a** and the other structures **4b–f** by use of various basis sets.

Calculation level	Conformational isomers for 4-*N*-cabamoyl-1-methylcytosine					
Basis set	**4a** *proximal*	**4b**	**4c**	**4d** *distal*	**4e**	**4f**
HF/6-31G*	**0**	+8.83	–2.32	**–3.48**	+12.05	–5.16
HF/6-31++G**	**0**	+7.97	–2.01	**–3.43**	+11.56	–4.76
MP2/6-31++G**	**0**	+7.53	–0.90	**–4.16**	+11.78	–4.23
B3LYP/6-31++G**	**0**	+6.17	–1.33	**–4.35**	+11.04	–4.84

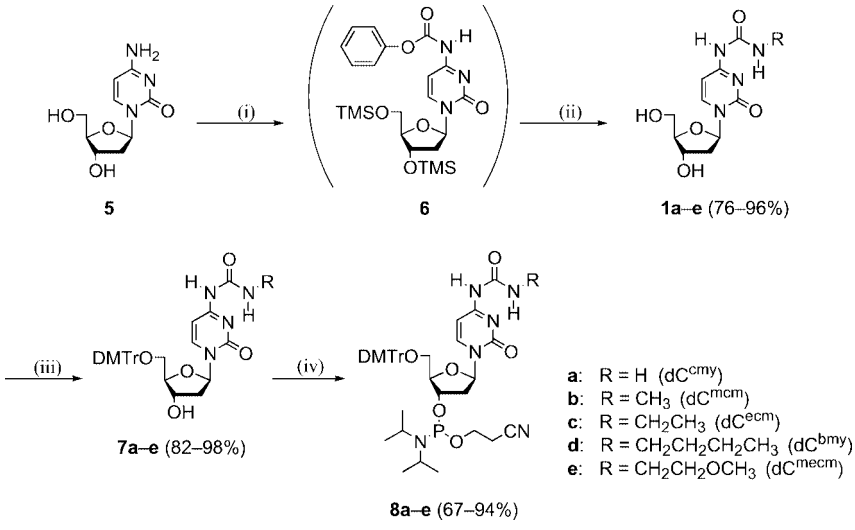
These isomeric structures were energy-optimized by DFT calculations at the B3LYP/6-31++G** level. In the structure **4a**, the carbonyl oxygen atom has the same orientation as that of the crystal structure of 4-*N*-acetylcytidine[14] and 4-*N*-[*N*-(methoxycarbonyl)carbamoyl]-3′,5′-di-*O*-acetyldeoxycytidine (**3**)[19] and the structure **4d** has the same orientation as that of the crystal structure of 4-*N*-ureidocarbonyldeoxycytidine (**2**).[19] In addition, the structure of **4f** has an intramolecular hydrogen bond similar to that of 4-*N*-benzoyl-5-methyldeoxycytidine,[21] which cannot have an intramolecular hydrogen bond similar to that of 4-*N*-acetyl-C because of the presence of the 5-methyl group. The values shown in the structures refer to the distance between the hydrogen-bonding atoms.

Compared with the energy of the conformational isomer **4a**, which is capable of formation of the Watson–Crick base pair, those of **4b–f** were evaluated by MO calculations at the HF/6-31G* and HF/6-31++G** levels in the gas phase (Table 1). The stabilization of these structures in the gas phase was ranked in the order **4f** > **4d** > **4c** > **4a** >> **4b** > **4e**. To see if higher calculations were necessary, these six structures were energy-optimized at the MP2/6-31++G** and B3LYP/6-31++G** levels. As the result, the order remained essentially unchanged. In all cases, the conformers **4f** and **4d** were the most and the second most stable and the difference in energy between them was very small. The isomers **4c** and **4f** are not found in the previous crystal structures,[19] so that the conformation of the 4-*N*-carbamoyl groups in solution must be studied in detail.

For more accurate conformational analysis of the conformers of 4-*N*-carbamoylcytosine, we synthesized dC^{cmy} (**1a**) and related compounds **1b–e** and studied their conformational analysis by use of ^1H and ^{13}C NMR spectroscopy. We also synthesized 4-^{15}N-labeled 4-*N*-carbamoyldeoxycytidine derivatives and 4-*N*-carbamoyl-5-methyldeoxycytidine derivatives for the study of tautomerism of the modified deoxycytidines.

Synthesis of 4-*N*-Carbamoyldeoxycytidine Derivatives

A general approach to synthesizing 4-*N*-(*N*-alkylcarbamoyl)deoxycytidine derivatives **1a–e** and their 3′-phosphoramidites **8a–e** is outlined in Scheme 1. The phenoxycarbonyl group was selectively introduced to the 4-amino function of deoxycytidine by the transient protection method using trimethylsilyl chloride.[22,23] Subsequently, the 4-*N*-(phenoxycarbonyl)deoxycytidine intermediate **6** was



Scheme 1. Reagents and conditions: (i) (A) TMSCl (4.0 equiv.), pyridine, room temp., 2 h, (B) phenyl chloroformate (1.5 equiv.), CH_2Cl_2/pyridine (1:1, v/v), room temp., 1 h; (ii) (A) RNH_2 (excess), pyridine, room temp., 2 h, (B) concd. NH_4OH, room temp., 2 h (for **1d**, **e**); (iii) DMTrCl (1.2 equiv.), pyridine, room temp., 4 h; (iv) (2-cyanoethoxy)bis(diisopropylamino)phosphane (1.1 equiv.), diisopropylamine (0.6 equiv.), 1*H*-tetrazole (0.6 equiv.), CH_2Cl_2, room temp., 6 h (for **8a**), chloro(2-cyanoethoxy)(diisopropylamino)phosphane (1.1 equiv.), ethyldiisopropylamine (1.3 equiv.), CH_2Cl_2, room temp., 30 min (for **8b–e**).

converted into various 4-*N*-carbamoyldeoxycytidine derivatives **1a**–**e** by treatment with the corresponding amines followed by removal of the trimethylsilyl groups. Treatment of **1a**–**e** with DMTrCl gave the 5′-*O*-dimethoxytritylated products **7a**–**e**. Compounds **7a**–**e** were converted into the phosphoramidite units **8a**–**e** by phosphitylation in the usual way.[24]

Conformational Studies of 4-*N*-Carbamoyldeoxycytidine Derivatives by Use of ^{1}H and ^{13}C NMR Spectroscopy

The ^{1}H NMR spectra of the 4-*N*-carbamoyldeoxycytidine derivatives were compared with those of 4-*N*-acetyldeoxycytidine and 4-*N*-methoxycarbonyldeoxycytidine.[3] Parthasarathy et al. reported that the X-ray crystal structure of 4-*N*-acetylcytidine, found in the first letter of the anticodon loop in various tRNAs, showed an intramolecular hydrogen bond between the carbonyl oxygen atom and the 5-vinyl proton.[14] Actually, the chemical shift of the 5-H proton of 4-*N*-acetylcytidine exhibited a downfield shift of 1.21 ppm in the ^{1}H NMR spectrum.[19] Recently, we have reported that acylation or alkoxycarbonylation of the 4-amino group of deoxycytidine does not affect the formation of the Watson–Crick base pair.[1–3] The chemical shifts of 5-H of 4-*N*-acyl- or 4-*N*-alkoxycarbonyl-modified deoxycytidines appear at ca. 1 ppm lower magnetic fields.[2,3]

The ^{1}H NMR spectra of deoxycytidine (**5**: dC), 4-*N*-acetyldeoxycytidine (**9**: dC^{ac}), 4-*N*-methoxycarbonyldeoxycytidine (**10**: dC^{moc}) and 4-*N*-carbamoyldeoxycytidine (**1a**: dC^{cmy}) in D_2O, are shown in Figure 5. The spectra of 4-*N*-carbamoyldeoxycytidine (**1a**) and its *N*-monosubstituted dC^{cmy} derivatives **1b**–**e** are similar to that of **5**. These results indicate that the 5-vinyl protons of **1a**–**e** are not involved in intermolecular hydrogen bonding so that their structures differ from those of 4-*N*-acyl- and 4-*N*-alkoxycarbonylcytosine base residues of **9** and **10** in D_2O. The structures of the 4-*N*-carbamoylcytosine moiety that can satisfy the above property are the second most and the most stable structures, **4d** and **4f**.

The solvent effect on the conformational change of the cmy group was also studied. Compound **1a** and its 3′,5′-*O*-disilylated derivative **11** were used. As shown in Figure 5, the ^{1}H NMR spectra of **1a** and **11** vary depending on the polarity of the solvent. The chemical shift of 5-H of **1a** in [D$_6$]DMSO is very similar to that observed in D_2O, except that the doublet peak is broader in [D$_6$]DMSO (data not shown). A more significant effect was observed when **11** was measured in the nonpolar solvent $CDCl_3$. Compared with the spectrum of **1a** in D_2O or [D$_6$]DMSO, the 5-H signal of **11** in $CDCl_3$ shows broadening with an apparent downfield shift ($\Delta\delta$ = 1.2 ppm). This result indicates that

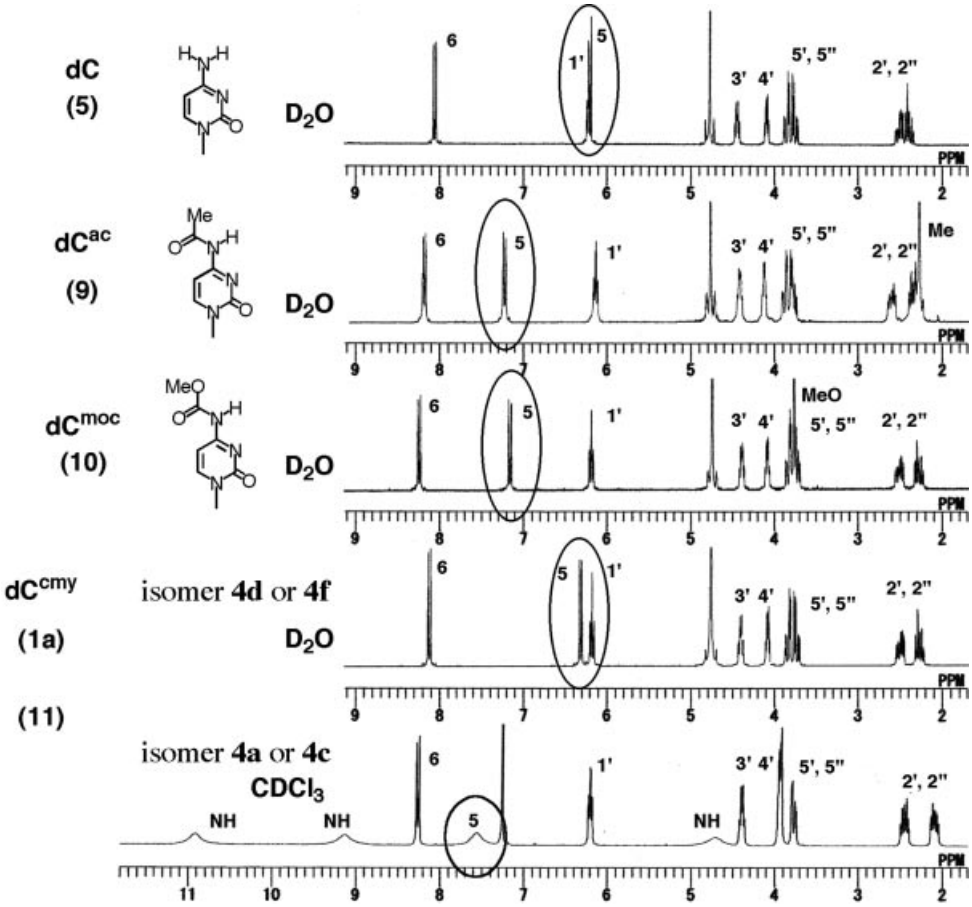


Figure 5. ^{1}H NMR spectra of deoxycytidine (dC, **5**), 4-*N*-acetyl-dC (dC^{ac}, **9**), 4-*N*-methoxycarbonyl-dC (dC^{moc}, **10**) and 4-*N*-carbamoyl-dC (dC^{cmy}) in D_2O and 3′,5′-*O*-bis-TBS-dC^{cmy} (**11**) in $CDCl_3$.

the conformation of the carbamoyl group in $CDCl_3$ is different from those in D_2O and [D_6]DMSO, suggesting the "*proximal*" conformation of **4a** is fixed by hydrogen bonding between 5-H and the carbamoyl group.

Tautomerism Studies of 4-*N*-Carbamoyldeoxycytidine Derivatives by Use of ^{15}N Labeling

Looking at the ^1H NMR spectra, it is still unclear whether the base moiety of **1a** exists in the 4-amino form **4a**, **4d** or the 4-imino form **4f**. To examine if dC^{cmy} (**1a**) exists in the tautomeric form **4f**, the 4-^{15}N-labeled deoxycytidine derivative **15** was also synthesized.[3,25] Thus, the 4-^{15}N-labeled species **15** was obtained from 3′,5′-*O*-bis(*tert*-butyldimethylsilyl)-2′-deoxyuridine (**12**) in an overall yield of 47%, as shown in Scheme 2. Compound **15** is useful to determine the tautomerism of the 4-*N*-carbamoylcytosine moiety because the amino tautomers such as **4a** and **4d** give a split 4-NH signal due to a large coupling constant (ca. 90 Hz) derived from a covalently bonded ^{15}N-^1H system, while the imino tautomers such as **4c** and **4f** having an unlabeled 3-NH proton give a singlet.

The ^1H NMR spectra of **14** in [D_6]DMSO and **15** in [D_6]DMSO and $CDCl_3$ are shown in Figure 6. We observed a split NH signal at δ = 7.18 (a), 9.72 (b), 10.91 (c) ppm and it can be assigned as the 4-NH proton of the cytosine base. From Figures 5 and 6, it was concluded that 4-*N*-carbamoyldeoxycytidine derivatives exist as amino tautomers with "*proximal*" conformation in $CDCl_3$ (**4a**) and "*distal*" conformation in [D_6]DMSO (**4d**). These results are consistent with the ^1H NMR analysis of 4-*N*-carbamoyl-5-methyldeoxycytidine derivatives shown in the Supporting Information.

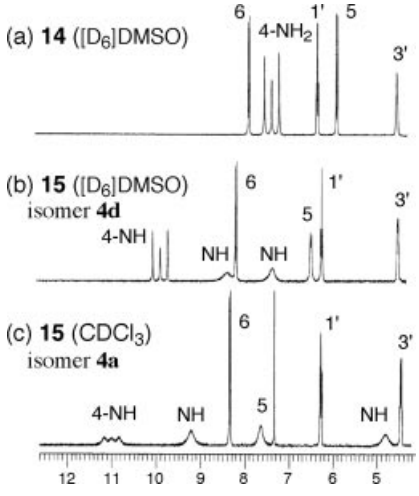


Figure 6. ^1H NMR spectra of 4-^{15}N labeled dC (**14**) in [D_6]DMSO (a) and 4-^{15}N labeled dC^{cmy} (**15**) in [D_6]DMSO (b) and $CDCl_3$ (c).

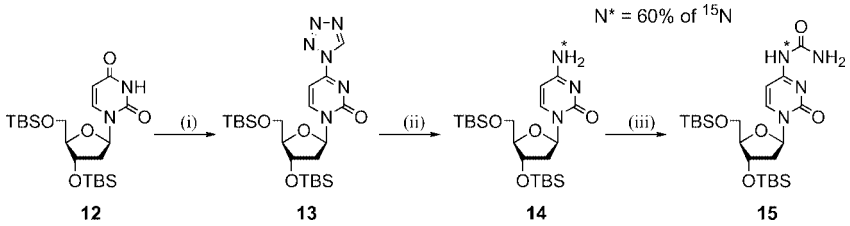
Effect of Solvent Polarity on the Dynamic Conformational Change between "*proximal*" and "*distal*"

Figure 7 shows the ^1H NMR spectra of **11** in $CDCl_3$ at various temperatures and solvents. The shape of the peaks assigned to 5-H and NH become sharper when the temperature is lowered to 243 K. The chemical shift of 5-H remains at δ = 7.58 ppm at 243 K and is identical to that measured at 298 K. Figure 7 (B) shows the ^1H NMR spectrum of **11** in the mixed solvent $CDCl_3$/MeOD in various ratios. The signal of 5-H is shifted upfield when the ratio of polar solvent (MeOD)/$CDCl_3$ is increased. Furthermore, when $CDCl_3$/MeOD (1:1, v/v) is used as the solvent, the shape of the 5-H peak changes to an ordinary doublet and its chemical shift is observed at δ = 6.26 ppm, identical to that of the spectrum measured in D_2O or [D_6]DMSO.

When the geometry of the cmy group of **11** is fixed to "*proximal*", as shown in Figure 2, the NH group of the cmy moiety can act as a donor so that compound **11** can have two donor sites and two acceptor sites to form four hydrogen bonds. Thus, in a nonpolar solvent such as $CDCl_3$ this does not inhibit the intermolecular hydrogen bonding; **11** would form a homodimer in an antiparallel direction with the four hydrogen bonds (Figure 7A), similar to the crystal structure found for the homodimer.[19] This solvent effect of the ^1H NMR spectra suggests that the dynamic conformational change between "*proximal*" and "*distal*" is due to the disruption of intermolecular hydrogen bonding by the addition of the polar solvent (MeOD).

Energy Profile of the Formation of a Watson–Crick Base Pair Evaluated by Ab Initio MO Calculations

The ^1H NMR study revealed that the solvent polarity significantly affects the conformation of the cmy group. The cmy group changes its geometry to form intermolecular hydrogen bonds in $CDCl_3$. We expected that a similar conformational change would occur when deoxyguanosine is located on the opposite side of the dC^{cmy} derivatives in a



Scheme 2. Reagents and conditions: (i) diphenyl phosphate (2 equiv.), 1*H*-tetrazole (2 equiv.), TsCl (2 equiv.), pyridine, room temp., 2 d; (ii) $^{15}NH_4Cl$ (1.2 equiv.), KOH (1.2 equiv.), Et_3N (1.4 equiv.), CH_3CN/H_2O (3:1, v/v), room temp., 24 h; (iii) (A) phenyl chloroformate (1.2 equiv.), pyridine (1.5 equiv.), CH_2Cl_2, room temp., 1 h, (B) pyridine/concd. NH_3 (1:1, v/v), room temp., 2 h.

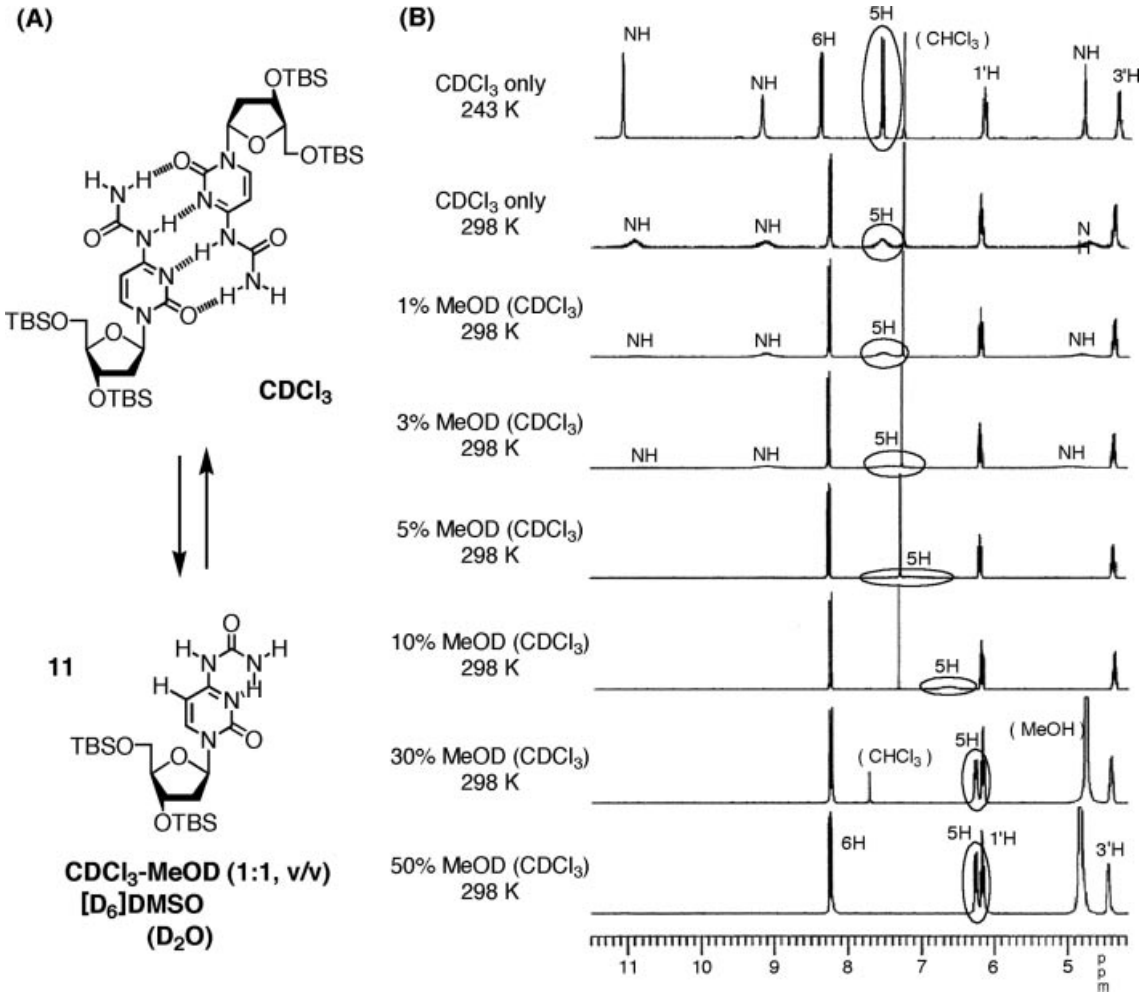


Figure 7. Solvent effect of 4-*N*-carbamoyldeoxycytidine on the intermolecular and intramolecular hydrogen-bonding formation.

DNA duplex. To estimate the energy of the formation of the Watson–Crick base pair between dC^{cmy} and dG, ab initio MO calculations of this base pair were carried out at the HF/6-31++G** level.

Although the structure **4d** in Figure 4 cannot form the Watson–Crick base pair, the structure **4a** can form three hydrogen bonds with the guanine base. The base-pair energy (Δ*E*) of 4-*N*-carbamoyl-1-methyl-C (**4a**) with 9-methyl-G was calculated by subtracting the energies of **4d** and 9-methyl-G from that of the base pair **4a**-G. The base pairing energies calculated at the HF/6-31++G** level are summarized in Figure 8. The C^{cmy}-G base pair has a stabilization energy of –27.4 kcal/mol, while the unmodified C-G base pair has a stabilization energy of –28.7 kcal/mol. Although there is energy loss due to the conformational change of the carbamoyl group, this energy loss is small enough to form a stable Watson–Crick base pair accompanying the conformational change from the structure **4d** to **4a**.

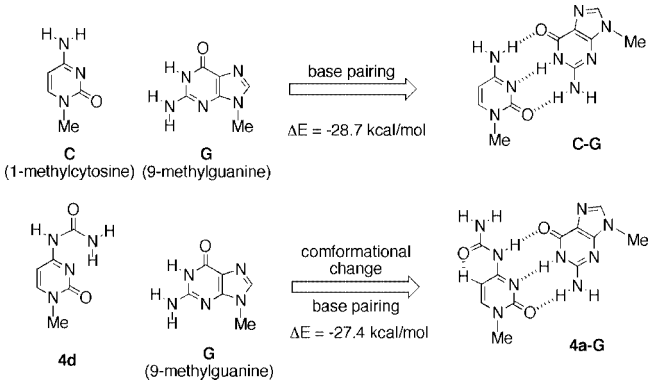


Figure 8. Predicted Watson–Crick base pair between 4-*N*-carbamoyl-dC and dG. The hydrogen-bonding energy (Δ*E*) including the energy loss of conformational change is also indicated.

Synthesis, Purification and Characterization of Oligodeoxyribonucleotides Containing Modified Bases

To evaluate the thermodynamic stability of the DNA duplexes incorporating these modified bases, they were incorporated into oligonucleotides. The solid-phase synthesis of oligodeoxyribonucleotides using a DNA/RNA synthesizer was carried out by use of the standard phosphoramidite method.[24,26] The oligomer was released from the polymer support and deprotected by treatment with concd. aq. NH_3 for 1 h. The product was purified on a C_{18}-cartridge by the

Table 2. Sequence of oligodeoxynucleotides containing 4-*N*-carbamoyl-dC derivatives.

Sequence	Isolated yield	MALDI-TOF mass		
		Found	Calcd	Molecular formula
d(TTTCTCC^{cmy}TTCTCT)	60%	3857.9	3857.6	$C_{126}H_{165}N_{32}O_{85}P_{12}^-$
d(TTTCTCC^{mcm}TTCTCT)	40%	3872.1	3871.7	$C_{127}H_{167}N_{32}O_{85}P_{12}^-$
d(TTTCTCC^{ecm}TTCTCT)	56%	3885.1	3885.7	$C_{128}H_{169}N_{32}O_{85}P_{12}^-$
d(TTTCTCC^{bcm}TTCTCT)	38%	3913.4	3913.7	$C_{130}H_{173}N_{32}O_{85}P_{12}^-$
d(TTTCTCC^{mecm}TTCTCT)	44%	3915.7	3915.7	$C_{129}H_{171}N_{32}O_{86}P_{12}$

DMTr-ON purification method and analyzed by anion-exchange HPLC and reversed-phase HPLC. The sequences and the isolated yields of the 13mers containing modified nucleosides are shown in Table 2. The composition of the purified products was confirmed by MALDI-TOF mass spectrometry.

Hybridization Properties of Oligodeoxynucleotides Containing Modified Bases

From the conformational studies of 4-*N*-carbamoyldeoxycytidine derivatives described above, it was predicted that these modified nucleosides would form stable Watson–Crick base pairs with deoxyguanosine.

The thermal stability of DNA duplexes containing a modified nucleoside was investigated in sodium phosphate buffer (pH = 7.0) containing 1.0 M NaCl. The melting curves of the duplexes containing 4-*N*-carbamoyldeoxycytidine derivatives are shown in Figure 9. The shapes of the melting curves were similar to that of the unmodified duplex. The *T*m values of duplexes containing 4-*N*-carbamoyl-dC derivatives are summarized in Table 3.

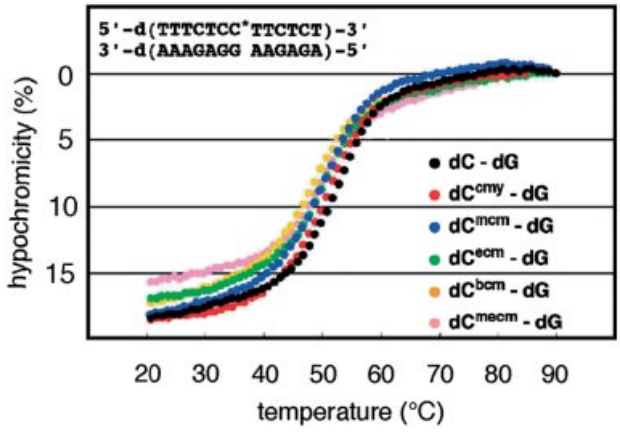


Figure 9. *T*m curves of 13mer duplexes containing a dC*-dG base pair.

As shown in Table 3, the *T*m value of the duplex containing 4-*N*-carbamoyldeoxycytidine (Entry 2: Y = G, 52.6 °C) was slightly lower by 0.6 °C than that of the unmodified duplex (Entry 1: Y = G, 53.2 °C). However, this *T*m value (Entry 2: Y = G, 52.6 °C) was not so low as that of the duplex containing a natural mismatched base pair (Entry 1:

Table 3. *T*m values[a] for DNA 13mer duplexes containing 4-*N*-carbamoyl-dC derivatives.

5'-d(TTTCTC**X**TTCTCT)-3'
3'-d(AAAGAG**Y**AAGAGA)-5'))

Entry	**X**	**Y** = G	Δ*T*m[b]	**Y** = T	**Y** = C	**Y** = A
1	C	53.2	--	37.8	30.7	32.8
2	C^{cmy} 	52.6	-0.6	38.9	34.6	37.8
3	C^{mcm} 	51.7	-1.5	39.6	32.5	38.1
4	C^{ecm} 	49.7	-3.5	38.8	32.4	38.8
5	C^{bcm} 	48.2	-5.0	38.0	36.1	40.3
6	C^{mecm} 	49.7	-3.5	37.3	30.6	37.4
7	*T*					49.2 (°C)

[a] The *T*m values are accurate within ±0.5 °C. The *T*m measurements were carried out in a buffer containing 10 mM sodium phosphate (pH = 7.0), 1.0 M NaCl, 0.1 mM EDTA and 2 μM duplex. [b] Δ*T*m is the difference in the *T*m value between the duplex having a modified base and that having a natural base.

Y = A, 32.8 °C; Y = C, 30.7 °C; Y = T, 37.8 °C). These results indicate that 4-*N*-carbamoyldeoxycytidine forms a stable base pair with deoxyguanosine. The duplex containing a C^{cmy}-G base pair (Entry 2: Y = G, 52.6 °C) is more stable than the duplex containing a T-A base pair (Entry 7: Y = A, 49.2 °C). Likewise, the *T*m values of duplexes containing monosubstituted 4-*N*-carbamoyldeoxycytidine derivatives (Entries 3–6) were relatively high, suggesting the formation of the Watson–Crick base pairs between modified nucleosides and deoxyguanosine.

It is likely that the carbamoyl groups of modified deoxycytidines change their orientation to the geometry that does not interrupt the formation of a Watson–Crick base pair with deoxyguanosine, as shown in Figure 10. The stability of the duplex decreases when the side chain length becomes longer, as shown in Entries 3–5 in Table 3. The results of Entries 5 and 6 suggest that incorporation of an oxygen atom into the alkyl side chain apparently increases the duplex stability (ΔTm = +1.5 °C). It is likely that this stabilization is caused by an increase in the hydrophilicity of the side chain that keeps the hydration structure in the major groove, essential for stabilization of the DNA duplex structure.[27] In the previous paper, we reported the effect of the length of the alkyl side chain of acyl or alkoxycarbonyl groups.[2,3] These results suggested that the hydrophobicity of the side chain of the carbamoyl group accounts for the duplex destabilization.

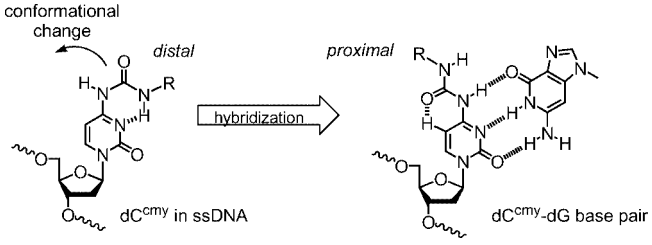


Figure 10. Conformational change of 4-*N*-carbamoyl-dC induced by duplex formation.

The *T*m values of DNA duplexes having a mismatched base pair (Y = T, C and A) are also summarized in Table 3. Compared with the *T*m values of all matched DNA duplexes (Y = G), the duplexes having a mismatched base pair were destabilized efficiently. The *T*m values of DNA duplexes containing a C*-A mismatch (Y = A) were somewhat higher by 4.6–7.5 °C than that having an unmodified mismatch base pair, but were at the same level as those of the duplexes containing a C*-T mismatch (Y = T) or a C-T mismatch. It should be noted that, among the modified deoxycytidine derivatives, C^{cmy} and C^{mecm} keep their G-recognition abilities against A, as shown by the ΔTm values (14.8 °C and 12.3 °C) observed between the modified and unmodified duplexes. From Table 3, it is likely that the modified deoxynucleoside derivatives **1a**–**e** can recognize A as a mismatched partner at the same level as T. Therefore, it seems that, when these modified deoxycytidines are incorporated into oligodeoxynucleotides, the modified bases can be used as the site capable of binding to a G base in the opposite strand without disturbing the duplex stability.

Conclusions

Modified oligodeoxyribonucleotides incorporating 4-*N*-carbamoyldeoxycytidine derivatives were synthesized. The ^{1}H NMR spectra of 4-*N*-carbamoyldeoxycytidine in D_2O suggests that the carbamoyl group forms an intramolecular hydrogen bond with the cytosine ring nitrogen atom. In contrast with 4-*N*-acyldeoxycytidines and 4-*N*-alkoxycarbonyldeoxycytidines, the orientation of the 4-*N*-carbamoyl group in D_2O is fixed at the geometry that inhibits the formation of the Watson–Crick base pair with a guanine base. However, it is interesting that in $CDCl_3$ 4-*N*-carbamoyl-dC derivatives exist as homodimers with four intermolecular hydrogen bonds, with a change in the geometry of the carbamoyl group. The *T*m analysis of oligodeoxynucleotides containing 4-*N*-carbamoyl-dC derivatives revealed that, in the process of their hybridization with the complementary oligodeoxynucleotides, the orientation of the carbamoyl group changes in a manner such that the stable Watson–Crick base pair can be formed with the guanine base.

Thus, we found that the geometry of 4-*N*-carbamoyldeoxycytidine derivatives is affected by the solvent and intermolecular hydrogen bonds. Their base-pairing abilities indicate that 4-*N*-carbamoyl groups might be useful as linkers between various functional residues and oligonucleotides when hydrophilic substitutents involving $-(CH_2CH_2O)_n-$ type spacers (see Entry 6 in Table 3), capable of maintaining the duplex structure without serious loss of the base-recognition ability, are attached to the nitrogen atom of the carbamoyl group. Further experiments with various 4-*N*-carbamoyl-modified deoxycytidine derivatives are under way to investigate the usefulness of these interesting inherent properties.

Experimental Section

General Remarks: All chemical reagents used are commercially available. Pyridine was distilled twice from *p*-toluenesulfonyl chloride and CaH_2 after being refluxed for several hours and stored over molecular sieves (4 Å). Triethylamine was distilled from CaH_2 and stored over molecular sieves (4 Å). TLC was performed using Merck Kieselgel 60 F_{254} precoated glass plates. Column chromatography was performed with silica gel C-200, C-300 (Wako Co. Ltd.), 60N (Kanto Chemical, Co., Inc.), NH (Fuji Silysia Chemical Ltd.) and a minipump for a goldfish bowl was conveniently used to attain sufficient pressure for rapid chromatographic separation. ^{1}H NMR spectra were recorded at 270 MHz and the chemical shifts were measured from the solvent peak as an internal standard (in $CDCl_3$ and [D_6]DMSO) or TSP (in D_2O) as an external standard. ^{13}C NMR spectra were recorded at 68 MHz and the chemical shifts were measured from the solvent peak as an internal standard. ^{31}P NMR spectra were recorded at 109 MHz and the chemical shifts were measured from 85% H_3PO_4 in $CDCl_3$ as an external standard. High-resolution ESI mass spectrometry was performed by use of a Mariner (PerSeptive Biosystems, Inc.).

General Procedure for the 4-*N*-Carbamoylation of Deoxycytidine Derivatives 1a–c

4-*N*-Carbamoyldeoxycytidine (1a): Deoxycytidine hydrochloride (791 mg, 3.0 mmol) was rendered anhydrous by repeated co-evapo-

ration with dry pyridine and finally dissolved in dry pyridine (30 mL). To the solution was added TMSCl (1.52 mL, 12.0 mmol) and the mixture was stirred at room temperature for 2 h. The mixture was diluted with dry CH_2Cl_2 (30 mL). Phenyl chloroformate (564 μL, 4.5 mmol) was added dropwise to the solution. After being stirred for 1 h, the mixture was diluted with $CHCl_3$ (30 mL). The $CHCl_3$ solution was washed three times with saturated $NaHCO_3$ (30 mL). The organic layer was collected, dried with Na_2SO_4, filtered and concentrated to dryness under reduced pressure. The residue was dissolved in pyridine/conc.NH_3 (1:1, v/v, 30 mL). After being stirred for another 2 h, the solution was concentrated to dryness under reduced pressure. The residue was dissolved in H_2O (30 mL) and washed three times with ethyl acetate (30 mL). The aqueous layer was concentrated under reduced pressure. The resulting precipitate was collected by filtration, washed with cold *i*PrOH and dried in vacuo to give **1a** (665 mg, 82%). ^{1}H NMR (D_2O): δ = 2.28–2.59 (m, 2 H), 3.73–3.90 (2 m, 2 H), 4.10–4.14 (m, 1 H), 4.41–4.46 (m, 1 H), 6.23 (dd, J = 6.4 Hz, 1 H), 6.41 (d, J = 7.4 Hz, 1 H), 8.13 (d, J = 7.4 Hz, 1 H) ppm. ^{13}C NMR (D_2O): δ = 44.7, 66.1, 75.3, 92.0, 92.1, 102.3, 148.6, 161.4, 161.7, 167.7 ppm. $C_{10}H_{14}N_4O_5$ (270.24): calcd. C 44.44, H 5.22, N 20.73; found C 44.19, H 5.31, N 20.78. ESIMS: calcd. for $C_{10}H_{15}N_4O_5$ $[M+H]^+$ 271.1042, found 271.1046.

4-*N*-(*N*-Methylcarbamoyl)deoxycytidine (1b):[28] Deoxycytidine hydrochloride (791 mg, 3.0 mmol) was allowed to react successively with TMSCl (1.52 mL, 12.0 mmol), phenyl chloroformate (564 μL, 4.5 mmol) and pyridine/40% aq. methylamine (1:1, v/v, 30 mL), as described for the synthesis of **1a**. The residue was dissolved in H_2O (30 mL) and washed three times with $CHCl_3$ (30 mL). The aqueous layer was concentrated in vacuo. The resulting precipitate was collected by filtration, washed with cold *i*PrOH and dried in vacuo to give **1b** (724 mg, 85%). ^{1}H NMR ([D$_6$]DMSO): δ = 2.00–2.26 (m, 2 H), 2.73 (2 m, 3 H), 3.49–3.57 (m, 1 H), 3.80–3.82 (m, 1 H), 4.18–4.20 (m, 1 H), 5.03 (t, J = 5.1 Hz, 1 H), 5.25 (d, J = 4.3 Hz, 1 H), 6.09 (dd, J = 6.3 Hz, 6.3 Hz, 1 H), 6.23 (d, J = 7.3 Hz, 1 H), 8.13 (d, J = 7.3 Hz, 1 H), 8.77 (br., 1 H), 9.89 (br., 1 H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 26.0, 40.6, 61.0, 70.0, 85.8, 87.7, 94.7, 143.3, 153.5, 154.2, 162.2 ppm. ESIMS: calcd. for $C_{11}H_{17}N_4O_5$ $[M+H]^+$ 285.1199, found 285.1197.

4-*N*-(*N*-Ethylcarbamoyl)deoxycytidine (1c): Deoxycytidine hydrochloride (791 mg, 3.0 mmol) was allowed to react successively with TMSCl (1.52 mL, 12.0 mmol), phenyl chloroformate (564 μL, 4.5 mmol) and pyridine/40% aq. ethylamine (1:1, v/v, 30 mL), as described for the synthesis of **1a**. The residue was redissolved in H_2O (30 mL) and washed three times with $CHCl_3$ (30 mL). The aqueous layer was concentrated in vacuo. The resulting precipitate was collected by filtration, washed with cold *i*PrOH and dried in vacuo to give **1c** (680 mg, 76%). ^{1}H NMR ([D$_6$]DMSO): δ = 1.08 (t, J = 7.1 Hz, 3 H), 1.92–2.03 (m, 1 H), 2.19–2.26 (m, 1 H), 3.16–3.24 (m, 2 H), 3.53–3.62 (m, 2 H), 3.80–3.82 (m, 1 H), 4.16–4.22 (m, 1 H), 5.00 (t, J = 5.1 Hz, 1 H), 5.22 (d, J = 4.3 Hz, 1 H), 6.09 (dd, J = 6.2 Hz, 6.3 Hz, 1 H), 6.24 (d, J = 7.1 Hz, 1 H), 8.13 (d, J = 7.1 Hz, 1 H), 8.85 (br., 1 H), 9.81 (br., 1 H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 15.1, 34.0, 40.7, 61.0, 70.0, 85.9, 87.8, 94.7, 143.3, 153.5, 153.6, 162.3 ppm. ESIMS: calcd. for $C_{12}H_{19}N_4O_5$ $[M+H]^+$ 299.1355, found 299.1352.

General Procedure for the 4-*N*-Carbamoylation of Deoxycytidine Derivatives 1d, e

4-*N*-(*N*-Butylcarbamoyl)deoxycytidine (1d): Deoxycytidine hydrochloride (791 mg, 3.0 mmol) was rendered anhydrous by repeated co-evaporation with dry pyridine and finally dissolved in dry pyridine (30 mL). To the solution was added TMSCl (1.52 mL, 12.0 mmol) and the mixture was stirred at room temperature for 2 h. The mixture was diluted with dry CH_2Cl_2 (30 mL). Phenyl chloroformate (564 μL, 4.5 mmol) was added dropwise to the solution. After being stirred for 1 h, the mixture was diluted with $CHCl_3$ (30 mL). The $CHCl_3$ solution was washed three times with saturated $NaHCO_3$ (30 mL). The organic layer was collected, dried with Na_2SO_4, filtered and concentrated under reduced pressure. The residue was dissolved in pyridine (30 mL) and to the mixture was added butylamine (889 μL, 9 mmol). After being stirred for another 2 h, concd. NH_3 (20 mL) was added to the mixture which was stirred for another 2 h. The residue was partitioned between H_2O (30 mL) and $CHCl_3$/pyridine (1:1, v/v, 50 mL). The organic layer was collected, dried with Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using 60N silica gel with CH_3Cl_3/MeOH to give the product **1d** (763 mg, 78%). ^{1}H NMR ([D$_6$]-DMSO): δ = 0.88 (t, J = 7.1 Hz, 3 H), 1.24–1.49 (m, 4 H), 3.13–3.20 (m, 2 H), 3.51–3.63 (m, 2 H), 3.80–3.82 (m, 1 H), 4.17–4.19 (m, 1 H), 5.03 (t, J = 5.2 Hz, 1 H), 5.25 (d, J = 4.2 Hz, 1 H), 6.09 (dd, J = 6.3 Hz, 6.4 Hz, 1 H), 6.23 (d, J = 6.5 Hz, 1 H), 8.13 (d, J = 6.5 Hz, 1 H), 8.97 (br., 1 H), 9.82 (br., 1 H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 13.9, 19.8, 31.6, 39.1, 40.9, 61.2, 70.2, 86.2, 87.9, 95.1, 143.3, 153.7, 153.9, 162.3 ppm. ESIMS; calcd. for $C_{14}H_{23}N_4O_5$ $[M+H]^+$ 327.1668, found 327.1666.

4-*N*-[*N*-(2-Methoxyethyl)carbamoyl]deoxycytidine (1e): Deoxycytidine hydrochloride (791 mg, 3.0 mmol) was allowed to react successively with TMSCl (1.52 mL, 12.0 mmol), phenyl chloroformate (564 μL, 4.5 mmol), 2-methoxyethylamine (782 μL, 9 mmol) and concd. NH_3 (20 mL), as described for the synthesis of **1d**. The residue was dissolved in H_2O (30 mL) and the aqueous solution was washed three times with $CHCl_3$ (30 mL). The aqueous layer was concentrated under reduced pressure. The resulting precipitate was collected by filtration, washed with cold ethyl acetate and dried in vacuo to give **1e** (976 mg, 96%). ^{1}H NMR ([D$_6$]DMSO): δ = 1.92–2.03 (m, 1 H), 2.18–2.29 (m, 1 H), 3.25 (s, 3 H), 3.30–3.43 (m, 4 H), 3.50–3.64 (m, 2 H), 3.81–3.83 (m, 1 H), 4.17–4.23 (m, 1 H), 5.00 (t, J = 5.2 Hz, 1 H), 5.22 (d, J = 4.0 Hz, 1 H), 6.09 (dd, J = 6.3 Hz, 6.5 Hz, 1 H), 6.27 (d, J = 6.8 Hz, 1 H), 8.14 (d, J = 6.8 Hz, 1 H), 8.91 (br., 1 H), 9.83 (br., 1 H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 39.0, 40.7, 58.0, 61.0, 70.0, 70.8, 85.9, 87.8, 94.6, 143.4, 153.5, 153.7, 162.2 ppm. ESIMS: calcd. for $C_{13}H_{21}N_4O_6$ $[M+H]^+$ 329.1461, found 329.1460.

General Procedure for the 5′-*O*-Dimethoxytritylation of Compounds 1a–e To Form Products 7a–e

4-*N*-Carbamoyl-5′-*O*-(4,4′-dimethoxytrityl)deoxycytidine (7a): Compound **1a** (540 mg, 2 mmol) was rendered anhydrous by repeated co-evaporation with dry pyridine and finally dissolved in dry pyridine (20 mL). To the solution was added DMTrCl (813 mg, 2.4 mmol) and the mixture stirred for 4 h. The mixture was diluted with $CHCl_3$ (30 mL) and the $CHCl_3$ solution was washed three times with saturated $NaHCO_3$ (30 mL). The organic layer was collected, dried with Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography eluting with CH_3Cl_3/MeOH containing 1% pyridine to give the product **7a** (938 mg, 82%). ^{1}H NMR ([D$_6$]DMSO): δ = 2.10 (m, 1 H), 2.29 (m, 1 H), 3.23 (m, 1 H), 3.73 (s, 6 H), 3.92 (m, 2 H), 4.25 (m, 1 H), 5.23 (d, J = 4.6 Hz, 1 H), 6.10 (dd, J = 6.0, 5.6 Hz, 1 H), 6.17 (d, J = 8.0 Hz, 2 H), 6.88 (d, J = 8.2 Hz, 4 H), 7.25–7.37 (m, 11 H), 7.95 (d, J = 8.0 Hz, 1 H), 9.75 (s, 1 H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 40.7, 63.0, 69.5, 85.6, 85.7, 94.7, 113.1, 126.6, 127.6, 129.6, 135.1, 135.2, 142.8, 144.1, 153.4, 154.1, 157.9, 162.2 ppm. $C_{31}H_{32}N_4O_7 \cdot 1/2H_2O$ (581.62): calcd. C 64.02, H

5.72, N 9.63; found C 64.33, H 5.82, N 9.30 (%). ESIMS: calcd. for $C_{31}H_{32}N_4NaO_7$ $[M+H]^+$ 595.2169, found 595.2170.

4-*N*-(*N*-Methylcarbamoyl)-5′-*O*-(4,4′-dimethoxytrityl)deoxycytidine (7b): Compound **1b** (426 mg, 1.5 mmol) was allowed to react successively with DMTrCl (610 mg, 1.8 mmol), as described for the synthesis of **7a**. Silica gel chromatography of the crude product with CH_3Cl_3/MeOH containing 1% pyridine gave the product **7b** (818 mg, 93%). ^{1}H NMR ($CDCl_3$): δ = 2.16–2.63 (m, 2 H), 2.82 (d, J = 3.2 Hz, 3 H), 3.38–3.52 (m, 2 H), 3.80 (s, 6 H), 4.06–4.09 (m, 1 H), 4.44–4.47 (m, 1 H), 6.24 (dd, J = 5.9, 5.9 Hz, 1 H), 6.83–6.86 (m, 4 H), 7.26–7.41 (m, 10 H), 8.03 (d, J = 6.1 Hz, 1 H), 8.89 (br., 1 H), 10.81 (br., 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 26.7, 41.5, 54.8, 62.6, 70.4, 86.2 86.4, 86.4, 96.4 (br.), 112.9, 126.6, 127.5, 127.6, 129.6, 134.9, 135.0, 142.2 (br.), 143.9 154.7 (br.), 155.6 (br.), 158.1, 164.0 (br.) ppm. ESIMS: calcd. for $C_{32}H_{35}N_4O_7$ $[M+H]^+$ 586.2506, found 586.2504.

4-*N*-(*N*-Ethylcarbamoyl)-5′-*O*-(4,4′-dimethoxytrityl)deoxycytidine (7c): Compound **1c** (298 mg, 1 mmol) was allowed to react successively with DMTrCl (407 mg, 1.2 mmol), as described for the synthesis of **7a**. Silica gel chromatography of the crude product with CH_3Cl_3/MeOH containing 1% pyridine gave the product **7c** (535 mg, 89%). ^{1}H NMR ($CDCl_3$): δ = 1.76 (t, J = 7.3 Hz, 3 H), 2.16–2.24 (m, 2 H), 2.26–2.63 (m, 1 H), 3.26–3.38 (m, 2 H), 3.39–3.53 (m, 2 H), 3.79 (s, 3 H), 3.80 (s, 3 H), 4.04–4.09 (m, 1 H), 4.42–4.52 (m, 1 H), 6.23 (dd, J = 5.9, 5.9 Hz, 1 H), 6.83–7.41 (m, 14 H), 8.02 (d, J = 7.6 Hz, 1 H), 8.89 (br., 1 H) 10.82 (br., 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.8, 34.9, 41.7, 55.2, 62.8, 70.9, 86.2, 86.6, 86.8, 96.9 (br.), 113.1, 113.3, 126.9, 127.8, 127.9, 129.8, 129.8, 142.2 144.0, 154.0 (br.), 156.1 (br.), 158.4, 164.4 (br.) ppm. ESIMS: calcd. for $C_{33}H_{37}N_4O_7$ $[M+H]^+$ 601.2657, found 601.2667.

4-*N*-(*N*-Butylcarbamoyl)-5′-*O*-(4,4′-dimethoxytrityl)deoxycytidine (7d): Compound **1d** (489 mg, 1.5 mmol) was allowed to react successively with DMTrCl (610 mg, 1.8 mmol), as described for the synthesis of **7a**. Silica gel chromatography of the crude product with CH_3Cl_3/MeOH containing 1% pyridine gave the product **7d** (924 mg, 98%). ^{1}H NMR ($CDCl_3$): δ = 0.91 (t, J = 7.2 Hz, 3 H), 1.32–1.57 (m, 4 H), 2.15–2.22 (m, 2 H), 2.55–2.61 (m, 1 H), 3.23–3.25 (m, 2 H), 3.37–3.51 (m, 2 H), 3.80 (s, 6 H), 4.05–4.09 (m, 1 H), 4.43–4.45 (m, 1 H), 6.22 (dd, J = 5.9, 5.9 Hz, 1 H), 6.83–6.86 (m, 4 H), 7.23–7.41 (m, 10 H), 8.02 (d, J = 7.7 Hz, 1 H), 8.84 (br., 1 H), 10.82 (br., 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 13.8, 20.1, 31.5, 39.7, 41.8, 55.1, 62.7, 70.9, 86.2, 86.6, 86.7, 113.1, 113.1 126.9, 127.8, 129.8, 135.1, 135.2, 142.1, 144.0, 154.0, 156.0, 158.3, 164.4 ppm. ESIMS: calcd. for $C_{35}H_{41}N_4O_7$ $[M+H]^+$ 629.2970, found 629.2977.

4-[*N*-(2-Methoxyethyl)carbamoyl]-5′-*O*-(4,4′-dimethoxytrityl)deoxycytidine (7e): Compound **1e** (492 mg, 1.5 mmol) was allowed to react successively with DMTrCl (610 mg, 1.8 mmol), as described for the synthesis of **7a**. Silica gel chromatography of the crude product with CH_3Cl_3/MeOH containing 1% pyridine gave the product **7e** (851 mg, 90%): ^{1}H NMR ($CDCl_3$): δ = 2.39–2.46 (m, 1 H), 2.50–2.56 (m, 1 H), 2.86 (s, 3 H), 3.32–3.46 (m, 6 H), 3.73 (s, 6 H), 3.92–4.02 (m, 1 H), 4.35–4.41 (m, 1 H), 6.16 (dd, J = 5.8, 5.9 Hz, 1 H), 6.72–6.79 (m, 4 H), 7.15–7.34 (m, 10 H), 7.95 (d, J = 7.6 Hz, 1 H), 8.99 (br., 1 H), 10.69 (br., 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 39.5, 41.6, 55.0, 58.4, 62.6, 70.6, 70.8, 86.1, 86.2, 86.5, 96.5 (br.), 112.9, 126.7, 127.6, 127.7, 128.1, 129.6, 135.0 135.1, 142.2, 143.8, 154.2 (br.), 155.7 (br.), 158.2, 163.8 (br.) ppm. ESIMS: calcd. for $C_{34}H_{39}N_4O_8$ $[M+H]^+$ 631.2768, found 631.2770.

4-*N*-Carbamoyl-5′-*O*-(4,4′-dimethoxytrityl)deoxycytidine 3′-*O*-(2-Cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (8a): Compound **7a** (1.73 g, 3.0 mmol) was rendered anhydrous by repeated co-evaporation with dry CH_3CN and finally dissolved in dry CH_2Cl_2 (30 mL). To the solution were added diisopropylamine (253 μL, 1.8 mmol), 1*H*-tetrazole (126 mg, 1.8 mmol) and (2-cyanoethoxy)bis(diisopropylamino)phosphane (1.05 mL, 3.3 mmol). The resulting mixture was stirred at room temperature for 6 h. The mixture was diluted with $CHCl_3$ (20 mL) and the $CHCl_3$ solution was washed three times with saturated $NaHCO_3$ (30 mL). The organic layer was collected, dried with Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography with hexane/ethyl acetate containing 0.5% triethylamine to give the product **8a** (2.18 g, 94%). ^{1}H NMR ($CDCl_3$): δ = 1.06–1.20 (m, 12 H), 2.21–2.31 (m, 1 H), 2.43–2.78 (m, 3 H), 3.36–3.89 (m, 12 H), 4.28–4.32 (m, 1 H), 4.53–4.69 (m, 1 H), 6.20–6.26 (m, 1 H), 6.81–6.88 (m, 4 H), 7.25–7.42 (m, 10 H), 8.08–8.22 (m, 1 H), 9.06 (br., 1 H), 10.85 (br., 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 20.2, 20.3, 20.3, 20.4, 24.5, 24.6, 24.7, 40.7, 40.7, 41.0, 41.1, 43.1, 43.1, 43.3, 43.3, 55.2, 55.2, 58.0, 58.1, 58.3, 58.4, 61.9, 62.3, 71.5, 71.7, 72.3, 72.5, 85.4, 85.5, 85.6, 86.6, 86.6, 86.7, 86.8, 97.1 (br), 113.1, 117.2, 117.3, 126.9, 127.0, 127.8, 128.0, 128.0, 129.9, 129.9, 129.9, 135.0, 135.1, 142.6, 143.9, 155.1, 156.0 (br.), 158.4, 164.4 (br.) ppm. ^{31}P NMR ($CDCl_3$): δ = 149.5, 149.9 ppm. ESIMS: calcd. for $C_{40}H_{50}N_6O_8P$ $[M+H]^+$ 773.3422, found 773.3441.

General Procedure for the Synthesis of 4-*N*-Modified Deoxycytidine 3′-Phosphoramite Derivatives 8b–e

4-*N*-(*N*-Methylcarbamoyl)-5′-*O*-(4,4′-dimethoxytrityl)deoxycytidine 3′-*O*-(2-Cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (8b): Compound **7b** (587 mg, 1.0 mmol) was rendered anhydrous by repeated co-evaporation with dry CH_3CN and finally dissolved in dry CH_2Cl_2 (10 mL). To the solution were added ethyldiisopropylamine (227 μL, 1.3 mmol) and chloro(2-cyanoethoxy)(diisopropylamino)-phosphane (245 μL, 1.1 mmol). The resulting mixture was stirred at room temperature for 30 min. The mixture was diluted with $CHCl_3$ (10 mL) and the $CHCl_3$ solution was washed three times with saturated $NaHCO_3$ (10 mL). The organic layer was collected, dried with Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography with hexane/ethyl acetate containing 0.5% triethylamine to give the product **8b** (527 mg, 67%). ^{1}H NMR ($CDCl_3$): δ = 1.16–1.29 (m, 12 H), 2.21–2.69 (m, 4 H), 2.73–2.75 (m, 3 H), 3.37–3.80 (m, 12 H), 4.20–4.25 (m, 1 H), 4.56–4.70 (m, 1 H), 6.25–6.28 (m, 1 H), 6.81–6.86 (m, 4 H), 7.25–7.42 (m, 10 H), 8.01–8.10 (m, 1 H), 8.90 (br., 1 H), 10.83 (br., 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 20.0, 20.1, 20.2, 20.3, 24.3, 24.4, 24.5, 24.6, 26.3, 40.6, 40.6, 40.9, 43.0 43.2, 43.2, 55.0, 57.9, 58.0, 58.2, 62.2, 62.4, 72.0, 72.3, 72.5, 72.7, 85.2, 85.3, 85.5, 85.6, 86.5, 86.6, 96.9, 113.0, 117.2, 117.2, 126.8, 127.7, 127.8, 127.9, 129.7, 129.7, 129.8, 129.8, 134.9, 134.9, 135.1, 135.1, 142.0, 143.8, 154.4, 156.1, 158.3, 164.2 ppm. ^{31}P NMR ($CDCl_3$): δ = 149.4, 150.0 ppm. ESIMS: calcd. for $C_{41}H_{52}N_6O_8P$ $[M+H]^+$ 787.3579, found 787.3584.

4-*N*-(*N*-Ethylcarbamoyl)-5′-*O*-(4,4′-dimethoxytrityl)deoxycytidine 3′-*O*-(2-Cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (8c): Compound **7c** (601 mg, 1.0 mmol) was allowed to react successively with ethyldiisopropylamine (227 μL, 1.3 mmol) and chloro(2-cyanoethoxy)(diisopropylamino)phosphane (245 μL, 1.1 mmol), as described for the synthesis of **8b**. Silica gel chromatography of the crude product with hexane/ethyl acetate containing 0.5% triethylamine gave the product **8c** (657 mg, 82%). ^{1}H NMR ($CDCl_3$): δ = 0.99–1.20 (m, 15 H), 2.10–2.17 (m, 1 H), 2.35–2.52 (m, 2 H), 2.52–2.67 (m, 1 H), 3.17–3.56 (m, 8 H), 3.70–3.73 (m, 6 H), 4.13–4.15 (m, 1 H), 4.46–4.57 (m, 1 H), 6.14–6.21 (m, 1 H), 6.71–6.78 (m, 5 H), 7.09–7.33 (m, 9 H), 7.91–8.00 (m, 1 H), 8.89 (br., 1 H), 10.76 (br., 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.7 20.2, 20.3, 20.3, 20.4,

24.5, 24.5, 24.6 24.7, 34.8, 40.8, 41.1, 41.1, 43.2, 43.3, 43.4, 55.2, 55.2, 58.1 58.2 58.4, 58.4, 62.4, 62.6, 72.3, 72.5, 72.8, 73.0 85.4, 85.5, 85.6, 85.7, 86.6, 86.7, 97.1, 113.1, 117.2, 117.3 126.9, 127.0, 127.8 128.0, 128.0, 129.8 129.9, 129.9, 130.0, 135.1, 135.1, 135.2, 142.0, 143.9, 153.9, 156.1, 158.4, 158.4 158.5 164.5 ppm. ^{31}P NMR ($CDCl_3$): δ = 149.5, 150.1 ppm. ESIMS: calcd. for $C_{41}H_{52}N_6O_8P$ $[M+H]^+$ 801.3741, found 801.3736.

4-*N*-(*N*-Butylcarbamoyl)-5′-*O*-(4,4′-dimethoxytrityl)deoxycytidine 3′-*O*-(2-Cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (8d): Compound **7d** (1.26 mg, 2.0 mmol) was allowed to react successively with ethyldiisopropylamine (453 μL, 2.6 mmol) and chloro(2-cyanoethoxy)(diisopropylamino)phosphane (491 μL, 2.2 mmol), as described for the synthesis of **8b**. Silica gel chromatography of the crude product with hexane/ethyl acetate containing 0.5% triethylamine gave the product **8d** (1.22 g, 74%). ^{1}H NMR ($CDCl_3$): δ = 0.89–1.52 (m, 19 H), 2.10–2.21 (m, 1 H), 2.41–2.68 (m, 3 H), 3.15–3.73 (m, 14 H), 4.13–4.19 (m, 1 H), 4.49–4.58 (m, 1 H), 6.16–6.21 (m, 1 H), 6.85–7.33 (m, 14 H), 7.95–8.03 (m, 1 H), 8.80 (br., 1 H), 10.81 (br., 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 13.8, 20.0, 20.2, 20.3, 24.4, 24.4, 24.5, 24.6, 31.3, 31.4, 39.6, 40.8, 41.1, 43.0, 43.1, 43.2, 43.3, 55.1, 55.1, 58.0, 58.3 62.2, 62.2 72.6 85.4, 85.6 86.5, 86.6, 97.1, 113.0, 117.2, 126.9, 127.7, 127.9, 127.9 129.8, 129.9, 135.0, 135.1, 135.2, 141.9, 143.8, 158.3 ppm. ^{31}P NMR ($CDCl_3$): δ = 149.3, 150.0 ppm. ESIMS: calcd. for $C_{41}H_{52}N_6O_8P$ $[M+H]^+$ 829.4054, found 829.4046.

4-*N*-[*N*-(2-Methoxyethyl)butylcarbamoyl]-5′-*O*-(4,4′-dimethoxytrityl)deoxycytidine 3′-*O*-(2-Cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (8e): Compound **7e** (631 mg, 1.0 mmol) was allowed to react successively with ethyldiisopropylamine (227 μL, 1.3 mmol) and chloro(2-cyanoethoxy)(diisopropylamino)phosphane (245 μL, 1.1 mmol), as described for the synthesis of **8b**. Silica gel chromatography of the crude product with hexane/ethyl acetate containing 0.5% triethylamine gave the product **8e** (711 mg, 86%). ^{1}H NMR ($CDCl_3$): δ = 1.04–1.18 (m, 12 H), 2.19–2.26 (m, 1 H), 2.42 (t, J = 6.3 Hz, 1 H), 2.58 (t, J = 6.3 Hz, 1 H), 2.66–2.72 (m, 1 H), 3.31–3.65 (m, 13 H), 3.72–3.80 (m, 6 H), 4.15–4.22 (m, 1 H), 4.51–4.65 (m, 1 H), 6.20–6.28 (m, 1 H), 6.80–6.88 (m, 4 H), 7.20–7.43 (m, 10 H), 8.01–8.12 (m, 1 H), 9.12 (br., 1 H), 10.85 (br., 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 20.0, 20.1, 20.1, 20.2, 24.3, 24.4, 24.5, 39.5, 40.7, 40.7, 40.9, 41.0, 43.0, 43.0, 43.2, 55.1, 58.0, 58.3, 58.5, 62.2, 62.5, 70.9, 72.0, 72.2, 72.6, 72.9, 85.4, 85.5, 85.6, 85.7, 86.5, 86.7, 97.1, 113.1, 117.3, 117.3, 127.0, 127.8, 128.0, 128.1, 129.9, 130.0, 135.1, 135.2, 135.3, 135.3, 142.2, 144.0, 154.3, 156.2, 158.5, 164.5 ppm. ^{31}P NMR ($CDCl_3$): δ = 149.4, 149.9 ppm. ESIMS: calcd. for $C_{41}H_{52}N_6O_8P$ $[M+H]^+$ 831.3846, found 831.3834.

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-4-*N*-carbamoyldeoxycytidine (11): 3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)deoxycytidine (456 mg, 1.0 mmol) was rendered anhydrous by repeated co-evaporation with dry pyridine and finally dissolved in dry CH_2Cl_2 (10 mL). To the solution were added pyridine (121 μL, 1.5 mmol) phenyl chloroformate (152 μL, 1.5 mmol) and the mixture was stirred at room temperature for 1 h. The mixture was diluted with $CHCl_3$ (10 mL) and the $CHCl_3$ solution was washed three times with saturated $NaHCO_3$ (15 mL). The organic layer was collected, dried with Na_2SO_4, filtered and concentrated under reduced pressure. Pyridine/concd.NH_3 (1:1, v/v, 10 mL) was added to the residue. After being stirred for another 2 h, the solution was concentrated to dryness. The residue was dissolved in $CHCl_3$ (15 mL), the $CHCl_3$ solution was washed three times with saturated $NaHCO_3$ (15 mL). The organic layer was collected, dried with Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography with hexane/$CHCl_3$ to give the product **11** (409 mg, 82%). ^{1}H NMR ([D$_6$]DMSO): δ = –0.05–0.03 (s, 12 H), 0.78–0.81 (m, 18 H), 2.03–2.11 (m, 1 H), 2.14–2.22 (m, 1 H), 3.61–3.79 (m, 3 H), 4.25–4.30 (m, 1 H), 6.01 (dd, J = 5.9 Hz, 5.9 Hz, 1 H), 6.26 (d, 1 H, 7.6 Hz), 7.12 (br., 1 H), 7.95 (d, J = 7.6 Hz, 1 H), 8.15 (br., 1 H), 9.66 (s, 1 H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = –5.6, –5.5, –5.0, –4.7, 17.7, 17.9, 25.6, 25.7, 40.5, 61.9, 70.7, 85.4, 86.8, 94.6, 142.6, 153.2, 154.0, 162.1 ppm. ESIMS: calcd. for $C_{22}H_{43}N_4O_5Si_2$ $[M+H]^+$ 499.2772, found 499.2773.

[4-^{15}N]-3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-4-*N*-carbamoyldeoxycytidine (15): [4-^{15}N]-3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-2′-deoxycytidine (**14**) (300 mg, 0.66 mmol) was rendered anhydrous by repeated co-evaporation with dry pyridine and finally dissolved in dry CH_2Cl_2 (7 mL). To the solution were added pyridine (80.7 μL, 1.0 mmol) and phenyl chloroformate (101 μL, 0.8 mmol) and the mixture was stirred at room temperature for 1 h. The mixture was diluted with $CHCl_3$ (7 mL) and the $CHCl_3$ solution was washed three times with saturated $NaHCO_3$ (10 mL). The organic layer was collected, dried with Na_2SO_4, filtered and concentrated under reduced pressure. Pyridine/concd. NH_3 (1:1, v/v, 8 mL) was added to the residue. After being stirred for another 2 h, the solution was concentrated to dryness under reduced pressure. The residue was dissolved in $CHCl_3$ (15 mL) and the $CHCl_3$ solution was washed three times with saturated $NaHCO_3$ (10 mL). The organic layer was collected, dried with Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography with hexane/$CHCl_3$ to give the product **15** (250 mg, 75%). ^{1}H NMR ([D$_6$]DMSO): δ = 0.06 (m, 12 H), 0.86 (m, 18 H), 2.07–2.19 (m, 1 H), 2.20–2.30 (m, 1 H), 3.68–3.87 (m, 3 H), 4.32–4.38 (m, 1 H), 6.07 (dd, J = 6.2, 5.6 Hz, 1 H), 6.31 (d, 1 H, 7.4 Hz), 7.19 (br., 1 H), 8.01 (d, J = 7.1 Hz, 1 H), 8.21 (br., 1 H), 9.72 (d, J_{HN} = 90.3 Hz, 1 H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = –5.6, –5.6, –5.0, –4.8, 17.6, 17.9, 25.6, 25.7, 40.5, 62.0, 70.8, 85.5, 86.9, 94.7, 142.8, 135.5, 154.2 (d, J_{CN} = 16.2 Hz), 162.4 (d, J_{CN} = 17.3 Hz) ppm. ESIMS: calcd. for $C_{22}H_{43}N_3{}^{15}NO_5Si_2$ $[M+H]^+$ 500.2742, found 500.2741.

Oligonucleotide Synthesis and Purification: All oligonucleotides were synthesized using an Applied Biosystems 392 oligonucleotide synthesizer on a 1 μmol scale, using PAC phosphoramidites (pac-A, isopropyl-pac-G and acetyl-C) from Glen Research. A 0.1 M solution of each modified nucleoside phosphoramidite was used. The standard pac-DNA phosphoramidite method was used for all the procedures described for deprotection and purification. After cleaving the oligonucleotides from the solid support under standard conditions (aq. NH_3, room temp., 1 h), DMTr-ON oligonucleotides were purified on a C_{18}-cartridge and analyzed by HPLC. HPLC was performed using the following systems. System A: Reversed-phase HPLC was performed using a Waters Aliance system with a Waters 3D UV detector and a μBondasphere 5 m C_{18} 100 Å column (Waters, 3.9×150 mm). A linear gradient (0–30%) starting from 0.1 M NH_4OAc and applying CH_3CN was used at a flow rate of 1 mL/min at 50 °C for 30 min. System B: Anion-exchange HPLC was performed using a Waters Aliance system with a Waters 3D UV detector and a Gen-Pak FAX column (Waters, 4.6×100 mm). A linear gradient (0–60%) starting from 25 mM sodium phosphate buffer (pH = 6.0) and applying 25 mM sodium phosphate buffer (pH = 6.0) containing 1 M NaCl (pH = 6.0) was used at a flow rate of 1 mL/min at 50 °C for 30 min. The MALDI-TOF mass spectrometry was carried out by use of a Voyager RP (PerSeptive Biosystems, Inc.).

Melting Temperature Analysis: A solution of an appropriate oligonucleotide and the complementary strand, both of which were arranged to be 2 μM, was prepared in 10 mM phosphate buffer (pH =

7.0) containing 1 M NaCl and 0.1 mM EDTA. The *T*m experiments were carried out using a Beckman DU-650 spectrophotometer. The solution containing oligonucleotides was kept at 80 °C for 10 min for complete dissociation of the duplex to form single strands, cooled at a rate of 0.5 °C/min and kept at 5 °C for 10 min. After that, the melting curves were determined at 260 nm using a UV spectrometer (Pharma Spec UV-1700, Shimadzu) by increasing the temperature at a rate of 0.5 °C. Each *T*m value was calculated by use of Igor Pro software (WaveMetrics, Inc.).

Theoretical Calculations: All ab initio MO calculations were carried out using the Gaussian 98 program[20] using a scalable supercomputer SGI Origin 2000.

Supporting Information (see footnote on the first page of this article): Tautomerism studies of 4-*N*-carbamoyldeoxycytidine derivatives by use of ^{13}C NMR chemical shifts.

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