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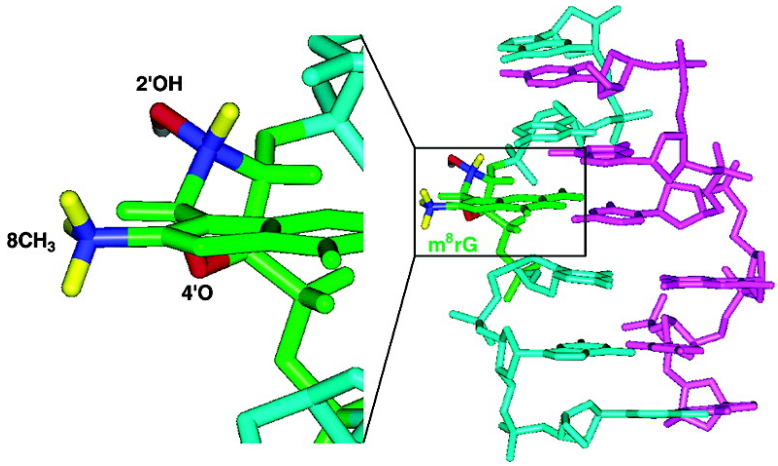
J. Am. Chem. Soc., **2003**, 125 (44), 13519-13524• DOI: 10.1021/ja036233i • Publication Date (Web): 14 October 2003



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8-Methylguanosine: A Powerful Z-DNA Stabilizer

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Abstract: Various modified guanine derivatives were synthesized and introduced into G_4 of $d(CGCGCG)_2$ to evaluate their capacity to stabilize Z-form DNA. It was found that the incorporation of 8-methylguanosine (m^8rG) in oligonucleotides stabilizes the Z form more dramatically than does the incorporation of 8-methyl-2′-deoxyguanosine (m^8G). This enhancement is ascribed to a reduction in the entropic penalty, which arises from the introduction of hydrophilic groups in solvent-exposed regions. The incorporation of m^8rG into DNA sequences markedly stabilizes the Z form even in the absence of NaCl. The Z-DNA stabilizer allows oligonucleotides with a wide range of sequences to be converted to the Z form. It could be a powerful tool for examining the molecular basis of many types of Z-form-specific reactions at the molecular level under physiological salt conditions.

Introduction

DNA is polymorphic and exists in a variety of distinct conformations.[1] Duplex DNA can adopt a variety of sequence-dependent secondary structures, which range from the canonical right-handed B form to the left-handed Z form.[2] Triplex and tetraplex structures also exist.[2b−d] All of these unique conformations are assumed to play important functional roles in gene expression by altering DNA−protein interactions.[3] Although Z-form DNA is one of the characteristic and significant local structures of DNA and has been extensively investigated in relation to transcription,[3a] the methylation of cytosine,[4] and the level of DNA supercoiling,[5] the biological function of Z-form DNA has not been well established. However, Rich and colleagues recently discovered that double-stranded RNA adenosine deaminase (ADAR1),[6] the tumor-associated protein DLM-1,[7] and the E3L protein of vaccina virus specifically bind to Z-form DNA.[8] Moreover, Liu and colleagues presented the first evidence that Z-DNA-forming sequences are required for chromatin-dependent activation of CSF1 promoter.[3d] Therefore, the possible biological roles of Z-DNA have drawn much current interest.[6c,7]

Although numerous studies have been made of Z-form DNA, its chemical properties are not well understood, presumably because of the difficulty in obtaining stable Z-form oligonucleotides under physiological salt conditions. We have demonstrated previously that the incorporation of a methyl group at the guanine C8 position (m^8G) stabilizes the Z form of oligonucleotides.[9] For example, the concentration of NaCl required to induce a B−Z transition is significantly reduced, with the midpoint NaCl concentrations for $d(CGCGCG)_2$ and $d(CGCm^8GCG)_2$ being 2600 and 30 mM, respectively. The development of a Z-stabilizing monomeric unit, the Z stabilizer, has allowed us to understand the solution structure of Z-DNA[9] and to identify the Z-form-specific C2′α-hydroxylation of the ^{I}U-containing Z-form $d(CGCG^IUGCG)/d(Cm^8GCACm^8GCG)$ duplex under UV irradiation.[10]

Although m^8G is a useful Z stabilizer, the strength of stabilization in an oligomer containing an AT base pair is inadequate. For example, the midpoint NaCl concentration for the B−Z transition of $d(CGCG^IUGCG)/d(Cm^8GCACm^8GCG)$ is 800 mM, which is substantially higher than physiological salt

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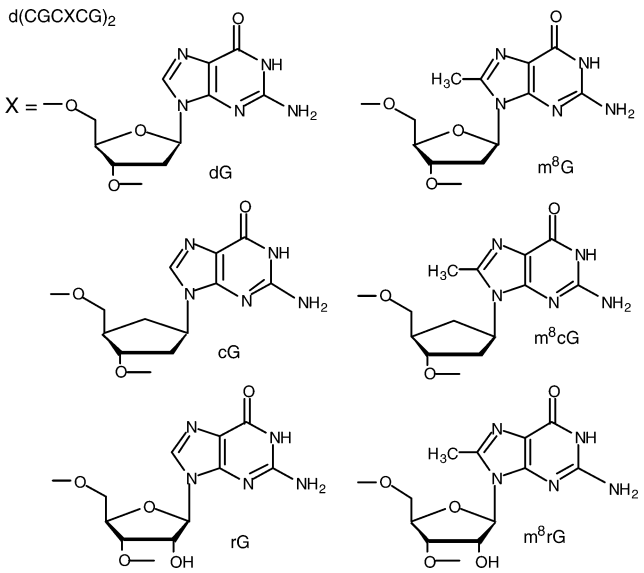
Scheme 1



concentrations. Moreover, the synthesis of m^8G monomer is carried out by the addition of methyl radicals under acidic conditions, during which depurination leads to a lowering of the yield. The design of more powerful and chemically stable new types of Z stabilizers is still desirable. Therefore, various modified guanine units that do not undergo depurination under acidic conditions were synthesized and introduced into G_4 of $d(CGCGCG)_2$ to evaluate their capacity to stabilize Z-form DNA (Scheme 1). It was found that incorporation of 8-methylguanosine (m^8rG) in DNA dramatically stabilizes the Z form even in the absence of NaCl, and facilitates the B−Z transition even for CA-containing sequences.

Results and Discussion

The syntheses of the 8-methyl-2′-deoxycarbaguanosine (m^8cG)- and m^8rG-containing oligonucleotides were carried out by phosphoramidite chemistry, beginning with 2-chloro-2′-deoxycarbainosine **1** and rG, respectively (Scheme 2). The monomeric unit, m^8cG, which possesses a cyclopentane ring in the sugar moiety, favors the syn conformation to a greater extent than does m^8G, as suggested by the ab initio MO calculation and experimentally confirmed by the nuclear Overhauser effect (NOE) intensity between C1′H and $8CH_3$ (Figure 1S, Supporting Information). The ab initio MO calculation at 6-31G* level indicates that the stabilization energies of the syn conformations of m^8cG and m^8G were 2.44 and 0.30 kcal/mol, respectively. CD spectroscopy is one of the more convenient methods used to study Z-DNA conformation.[11] In B-DNA, a positive Cotton effect appears at 280 nm, and a more intense negative band appears at 295 nm in Z-DNA.[11] Thus, the CD spectrum was used to monitor the conformational state at various NaCl concentrations, and the results are shown in Figure 1. Unexpectedly, when m^8cG was incorporated, $d(CGCm^8cGCG)_2$ did not convert as effectively to the Z form as did $d(CGCm^8GCG)_2$: the midpoint NaCl concentration for $d(CGCm^8cGCG)_2$ was 1300 mM, although it is lower than that for the unmodified duplex (2600 mM) (Table 1). The proportions of Z, B, and SS for these hexamers as a function of temperature are shown in Figure 2a−g. Thermodynamic parameters were obtained by the van't Hoff plot for B−Z transition (Figure 2h), and the results are summarized in Table 1. Although methyl substitution results in a favorable enthalpic effect (13.4 and 14.2 kcal/mol vs 11.7 kcal/mol) on the stability of Z-DNA, an unfavorable entropic effect is also induced by the CH_2 group at the 6′ position and the methyl group at the C8 position (44.6 and 43.9 vs 42.3 cal mol^{-1} K^{-1}). Inspection of the molecular model of $d(CGCm^8cGCG)_2$ suggests that the C6′ position of the m^8cG residue is exposed to solvent (Figure 3a), which causes a large entropical penalty (2.3 cal mol^{-1} K^{-1}), as observed in the m^8G residue (1.6 cal mol^{-1} K^{-1}), relative to that of $d(CGCGCG)_2$. Accordingly, 2′-deoxycarbaguanosine(cG)-incorporated $d(CGCcGCG)_2$ did not undergo transition to the Z form at all, even in the presence of 5 M NaCl (Table 1 and Figure 1c). These results suggest the intriguing possibility that the introduction of a hydrophilic group to the solvent-exposed site stabilizes the Z form (Figure 3b). In fact, incorporation of 8-methylguanosine (m^8rG) possessing a C2′ hydroxyl group and an O4′ oxygen into the hexamer greatly stabilized the Z form: $d(CGCm^8rGCG)_2$ showed a CD spectrum typical of the Z form, even in the absence of NaCl (Figure 1b). The thermodynamic parameters of the B−Z transition clearly indicate that a reduction in entropy (−1.9 cal mol^{-1} K^{-1}) largely contributes to the increased stabilization by m^8rG as compared to that by m^8G, which arises from the introduction of hydrophilic groups in solvent-exposed regions. Furthermore, at physiological salt concentrations, the reduction in the entropic penalty is more clearly observed (Table 1). In fact, incorporation of guanosine (rG) possessing a hydrophilic group itself stabilized the Z form;[12] the midpoint for $d(CGCrGCG)_2$ containing an rG was 800 mM (Table 1). In addition to hydrophilic stabilization, the preferred C3′ *endo* conformation of ribose also contributes to the dramatic stabilization of the Z form by m^8rG.

Significant stabilization of the Z form by incorporation of m^8rG was observed in duplex **c** containing an A^IU base pair, which showed the typical Z form only in 5 mM sodium cacodylate buffer. The CD spectra of duplex **c** under various NaCl concentrations are shown in Figure 4a. Furthermore, the effect of stabilization was confirmed in three AT-base-pair-containing octanucleotides: the midpoint NaCl concentration for duplex **e** was 200 mM, which is a more than 10-fold reduction relative to the corresponding deoxyoctanucleotide **d** (Table 2). The results indicate that the incorporation of m^8rG into just one strand is capable of stabilizing the Z-DNA. The results of **f** and **g** clearly demonstrate that a single m^8rGC base pair is enough to stabilize Z-DNA.

To demonstrate the usefulness of m^8rG as a Z-DNA stabilizer, photoreaction of ^IU-containing Z-form deoxyoctanucleotide was examined. Under 302 nm irradiation, duplex **c** in the presence of 50 mM NaI underwent efficient Z-form-specific C2′α-hydroxylation (>90%, based on consumed octamer) without the halogen exchange reaction observed during the photoirradiation of duplex **a** in 2 M NaCl (Figure 4b).[10] Interestingly, the C2′α-hydroxylated product (2′OH) stabilized the Z form: the midpoint

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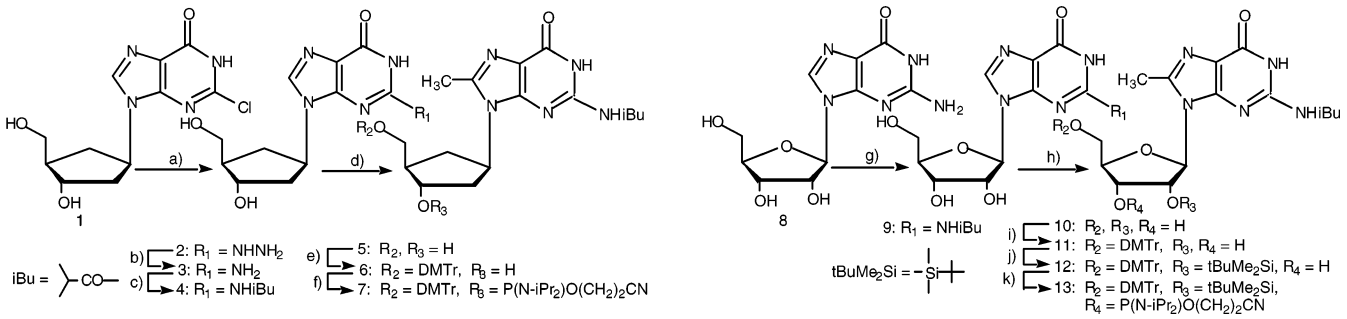
Scheme 2 [a]



[a] (a) NH_2NH_2, CH_3OH; (b) H_2, PtO_2; (c) pyridine, TMSCl, isobutyric anhydride; (d) H_2SO_4, $FeSO_4 \cdot 7H_2O$, *tert*-butylhydroperoxide; (e) pyridine, DMAP, Et_3N, DMTrCl; (f) $NC(CH_2)_2OP(Cl)N(^iPr)_2$, $(^iPr)_2NH$, CH_2Cl_2; (g) pyridine, TMSCl, isobutyric anhydride; (h) H_2SO_4, $FeSO_4 \cdot 7H_2O$, *tert*-butylhydroperoxide; (i) pyridine, DMAP, Et_3N, DMTrCl; (j) *tert*-butyldimethylsilyl chloride, dichloroethane, imidazole; (k) $NC(CH_2)_2OP(Cl)N(^iPr)_2$, $(^iPr)_2NH$, CH_2Cl_2.

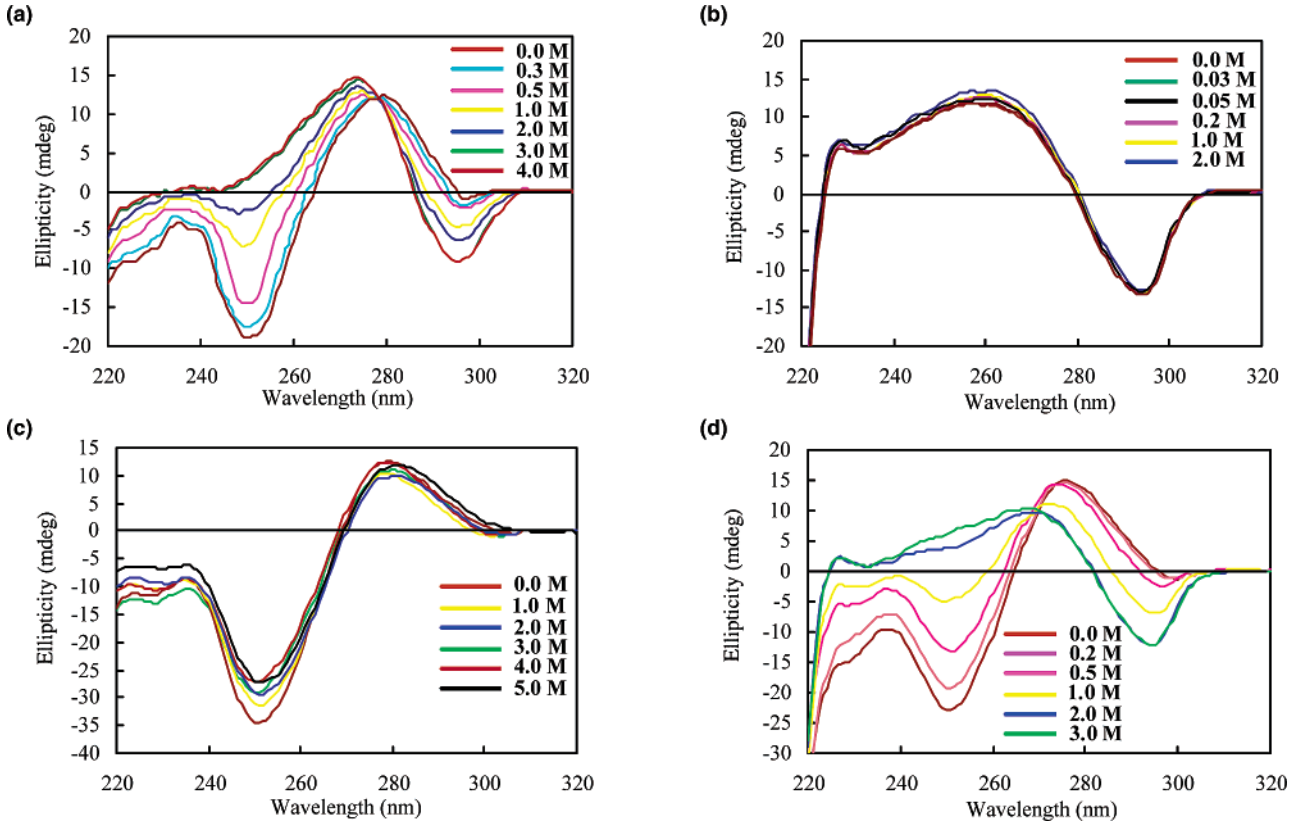


Figure 1. CD spectra of $d(CGCm^8cGCG)_2$ (a), $d(CGCm^8rGCG)_2$ (b), $d(CGCcGCG)_2$ (c), and $d(CGCrGCG)_2$ (d) in 5 mM Na cacodylate buffer, pH 7.0, at 10 °C at various NaCl concentrations (all oligomers, 0.15 mM base concentration).

NaCl concentrations for **a** and **b** were 800 and 420 mM, respectively (Table 2). It is interesting to note that a change in the B−Z equilibrium induced by C2′α-hydroxylation might represent a new type of damaging mechanism in 5-halouracil-containing DNA under UV irradiation.

In conclusion, the present study demonstrates that the incorporation of m^8rG in oligonucleotides dramatically stabilizes the Z form, mainly by a reduction in the entropic penalty, which arises from the introduction of hydrophilic groups in solvent-exposed regions. We have shown that m^8rG is a much better Z stabilizer than the previously used m^8G, in that it allows oligonucleotides with a wide range of sequences to be converted to the Z form. Incorporation of the m^8rG moiety into DNA oligomers could be a powerful tool with which to examine the molecular basis for many types of Z-form-specific reactions at the molecular level under physiological salt conditions.

Experimental Section

General. 2-Chloro-2′-deoxycarbaguanosine (**1**) was obtained from Takeda Chemical Industries. ^1H NMR (500 MHz, DMSO-d_6): δ 8.04 (s, 1H, H-8), 4.91 (m, 1H, 1′), 4.07 (m, 1H, 3′), 3.46 (m, 2H, 5′), 2.33 (m, 1H, 6′), 2.15 (m, 1H, 2′), 2.01 (m, 2H, 2″ and 4′), 1.65 (m, H, 6″). Reactions were monitored by thin-layer chromatography (TLC) using 0.25 mm silica gel 60 plates impregnated with 254 nm fluorescent indicator (purchased from Merck). Column chromatography was performed on Acros Chimica silica gel (0.2−0.5 mm, pore size 4 nm). The NMR spectra were recorded on a JEOL JNM-A 500 magnetic resonance spectrometer. Calf intestine alkaline phosphatase (AP) and P1 nuclease were purchased from Boehringer Mannheim. The following abbreviations apply to spin multiplicity: s (singlet), d (doublet), m (multiplet), and b (broad). Fast atom bombardment mass spectra (FABMS) were recorded on a JEOL-JMS-AX505H mass spectrometer. Electrospray ionization mass spectra (ESI-MASS) were recorded on a PE Sciex API 165 mass spectrometer.

Table 1. Midpoint NaCl Concentrations[a] and Thermodynamic Parameters[b] for the B−Z Transition in Modified d(CGCGCG)$_2$ Derivatives[c]

duplexes	NaCl (mM)	ΔG^{297K} (kcal mol^{-1})	ΔH (kcal mol^{-1})	ΔS (cal mol^{-1} K^{-1})
d(CGCGCG)$_2$	2600	−0.86 ± 0.05	11.7 ± 1.0	42.3 ± 1.2
d(CGCm^{8}GCG)$_2$	30	1.16 ± 0.10	14.2 ± 1.3	43.9 ± 1.5 (1.6)[d]
		0.24 ± 0.04	10.1 ± 0.5	37.2 ± 0.9[f]
d(CGCm^{8}cGCG)$_2$	1300	0.15 ± 0.03	13.4 ± 0.7	44.6 ± 1.7 (2.3)
d(CGCcGCG)$_2$	>5000	nd[e]	nd	nd
d(CGCm^{8}rGCG)$_2$	0	1.32 ± 0.13	13.8 ± 0.8	42.0 ± 1.3 (−0.3)
		0.42 ± 0.05	9.3 ± 0.6	33.1 ± 0.7[f]
d(CGCrGCG)$_2$	800	0.52 ± 0.04	13.0 ± 0.8	41.8 ± 0.9 (−0.5)

[a] Midpoint NaCl was determined from CD measurements at 10 °C at various NaCl concentrations. The B−Z conformational transition was analyzed by the data collected below 25 °C. [b] Thermodynamic parameters were calculated by plotting $\ln(P_Z/P_B)$ versus $1/T$ in 3.0 M NaCl, 5 mM Na-cacodylate buffer, pH 7.0. [c] All experiments were carried out using 0.15 mM hexanucleotide (base concentration) in 5 mM Na-cacodylate buffer, pH 7.0. [d] The numbers in parentheses are the difference in ΔS relative to d(CGCGCG)$_2$. [e] These data were not determined. [f] These data were determined in the physiological salt concentrations (10 mM NaCl, 100 mM KCl).

2-Hydrazino-2′-deoxycarbaguanosine (2). The 2-chloro-2′-deoxycarbaguanosine (**1**) (3.0 g, 10.1 mmol) was added to methanol (30 mL). To this solvent was added hydrazine hydrate (0.6 g, 12.3 mmol). The mixture was refluxed gently for 24 h, and then solvent was removed in a vacuum, and the residue was triturated with ethanol (100 mL). The resulting white precipitate was filtered to give 2-hydrazine-2′-deoxycarbainosine (2.1 g), yield 74%. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.24 (s, NH), 4.85 (m, 1H, 1′), 4.07 (m, 1H, 3′), 3.46 (m, 2H, 5′), 2.27 (m, 1H, 6′), 2.14 (m, 1H, 2′), 1.98 (m, 2H, 2″ and 4′), 1.64 (m, 1H, 6″). FABMS, *m*/*e* calcd for $C_{11}H_{16}N_6O_3$ (M + H) 281.0, found 281.1.

2′-Deoxycarbaguanosine (3). The 2-hydrazino-2′-deoxycarbainosine (1.0 g, 3.6 mmol) was dissolved in a solution which was a mixture of methanol (40 mL) and distilled water (10 mL). To this mixture was added PtO_2 (0.3 g). The mixture was hydrogenated for 40 h at 55 °C under H_2. After completion of the hydrogenation, the catalyst was removed using a filter aid and washed with a methanol−water mixture. The filtrate was concentrated to give the 2′-deoxycarbaguanosine (0.7 g) as a white powder, which was recrystallized from water (yield 70%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.78 (s, 1H, H-8), 4.81 (m, 1H, 1′), 4.06 (m, 1H, 3′), 3.44 (m, 2H, 5′), 2.27 (m, 1H, 6′), 2.10 (m, 1H, 2′), 1.96 (m, 2H, 2″ and 4′), 1.58 (m, 1H, 6″). FABMS, *m*/*e* calcd for $C_{11}H_{15}N_5O_3$ (M + H) 266.0, found 266.0.

***N*^{2}-Isobutyryl-2′-deoxycarbaguanosine (4).** To 0.6 g (2.2 mmol) of 2′-deoxycarbaguanosine dried three times by evaporation of pyridine (10 mL) and suspended in 8 mL of dry pyridine was added 3.2 mL (25 mmol) of trimethylchlorosilane. After the solution was stirred for 30 min, 4.1 mL (25 mmol) of isobutyric anhydride was added, and the mixture was stirred for 3 h at room temperature under N_2. The reaction was cooled in an ice bath, and 10 mL of water was added. After 5 min, 10 mL of 29% aqueous ammonia was added, and the reaction was stirred for 15 min. The solution was then evaporated to near dryness, and the residue was dissolved in 25 mL of water. The solution was washed once with 25 mL of ethyl acetate/ether (1:1). The organic layer was extracted with 12.5 mL of water, and the combined aqueous layers were concentrated to about 15 mL. Crystallization occurred quickly. The target compound (0.5 g) was given by concentration of the filtrate (yield 69%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.12 (s, 1H, H-8), 4.92 (m, 1H, 1′), 4.08 (m, 1H, 3′), 3.45 (m, 2H, 5′), 2.77 (m, 1H, isobutyryl CH), 2.34 (m, 1H, 6′), 2.15 (m, 1H, 2′), 2.01 (m, 2H, 2″ and 4′), 1.62 (m, 1H, 6″), 1.12 (d, 6H, J = 6.5 Hz, 2CH$_3$). FABMS, *m*/*e* calcd for $C_{15}H_{15}N_5O_3$ (M + H) 336.1, found 336.3.

8-Methyl-*N*^{2}-isobutyryl-2′-deoxycarbaguanosine (5). To a solution of **4** (0.5 g, 1.5 mmol) and $FeSO_4 \cdot 7H_2O$ (3.4 g, 12 mmol) in 80 mL of 1 N H_2SO_4 was added an aqueous solution (50 mL) containing 1.3 mL of 70% *tert*-butyl hydroperoxide (9.5 mmol) dropwise over a period of 5 min. After being stirred at 0 °C for 60 min, the reaction mixture was neutralized with saturated KOH solution. The supernatant obtained by centrifugation resulting in a brownish solid was triturated three times with 100 mL of methanol. The combined methanol solution was concentrated, and the residue was subjected to silica gel column chromatography. Elution with CH_2Cl_2/methanol (9:1) afforded 8-methyl-*N*-isobutyryl-2′-deoxycarbaguanosine (**5**) as a white powder, yield 0.37 g (71%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 4.90 (m, 1H, 1′), 4.10 (m, 1H, 3′), 3.55 (m, 2H, 5′), 2.76 (m, 1H, isobutyryl CH), 2.35 (m, 1H, 6′), 2.16 (m, 1H, 2′), 2.01 (m, 2H, 2″ and 4′), 1.87 (s, 3H, −8CH$_3$), 1.61 (m, 1H, 6″), 1.12 (d, 6H, J = 6.5 Hz, 2CH$_3$). ESMS, *m*/*e* calcd for $C_{16}H_{23}N_5O_4$ (M + H) 350.3, found 350.5.

8-Methyl-*N*^{2}-isobutyryl-5′-*O*-(dimethoxytrityl)-2′-deoxycarbaguanosine (6). To 0.3 g (0.9 mmol) of **5** dried three times by evaporation of pyridine (10 mL) and suspended in 8 mL of dry pyridine were added 0.5 g (1.3 mmol) of 4,4′-dimethoxytrityl chloride, 0.2 mL (1.3 mmol) of triethylamine, and 2.8 mg (0.03 mmol) of 4-dimethlyaminopyridine. After it stood overnight, the reaction was cooled in an ice bath, and 10 mL of 5% $NaHCO_3$ was added. The mixture was extracted twice with 60 mL of ethyl acetate. The organic layer was evaporated to dryness, and the target compound was given by silica gel column chromatography: 0.4 g, yield was 67%. ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.20−7.39 (m, 9H, ph), 6.89 (m, 4H, ph), 4.96 (m, 1H, 1′), 4.16 (m, 1H, 3′), 3.74 (s, 6H, 2CH$_3$), 3.18 (m, 2H, 5′), 3.02 (m, 1H 6′), 2.77 (m, 1H, isobutyryl CH), 2.14 (m, 1H, 2′), 2.05 (m, 2H, 2″ and 4′), 1.88 (s, 3H, −8CH$_3$), 1.62 (m, 1H, 6″), 1.10 (d, 6H, J = 6.5 Hz, 2CH$_3$). ESMS, *m*/*e* calcd for $C_{37}H_{41}N_5O_6$ (M + H) 652.0, found 652.1.

***N*^{2}-Isobutyrylguanosine (9).** To 7 g (25 mmol) of guanosine dried three times by evaporation of pyridine (100 mL) and suspended in 140 mL of dry pyridine was added 17.5 mL (125 mmol) of trimethylchlorosilane. After the solution was stirred 2 h, 21 mL (125 mmol) of isobutyric anhydride was added, and the mixture was stirred for 4 h at room temperature under N_2. The reaction was cooled in an ice bath, and 35 mL of water was added. After 15 min, 35 mL of 29% aqueous ammonia was added, and the reaction was stirred for 15 min. The solution was then evaporated to near dryness, and the residue was dissolved in 500 mL of water. The mixture was extracted twice with 200 mL of CH_2Cl_2. The organic layer was evaporated to drynesss, and the target compound was given by silica gel column chromatography: 3.6 g, yield was 39%. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.24 (s, 1H, H-8), 5.79 (m, 1H, 1′), 4.42 (m, 1H, 2′), 4.08 (m, 1H, 3′), 3.81 (m, 1H, 4′), 3.54 (m, 2H, β5′), 2.77 (m, 1H, isobutyryl CH), 1.12 (d, 6H J = 7.0 Hz, 2CH$_3$). ESMS, *m*/*e* calcd for $C_{15}H_{21}N_5O_6$ (M + H) 354.3, found 354.5.

8-Methyl-*N*^{2}-isobutyrylguanosine (10). To a solution of *N* ^{2}-isobutyrylguanosine (1 g, 2.6 mmol) and $FeSO_4 \cdot 7H_2O$ (6.7 g, 24.1 mmol) in 160 mL of 1 N H_2SO_4 was added dropwise an aqueous solution (100 mL) containing 2.6 mL of 70% *tert*-butyl hydroperoxide (9.5 mmol) over a period of 5 min. After being stirred at 0 °C for 2 h, the reaction mixture was neutralized with saturated KOH solution. The supernatant obtained by centrifugation resulting in a brownish solid was triturated three times with 100 mL of methanol. The combined methanol solution was concentrated, and the residue was subjected to silica gel column chromatography. Elution with CH_2Cl_2/methanol (9:1) afforded 8-methyl-*N* ^{2}-isobutyrylguanosine (**10**) as a white powder: yield 0.6 g (59%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 5.81 (m, 1H, 1′), 4.62 (m, 1H, 2′), 4.12 (m, 1H, 3′), 3.82 (m, 1H, 4′), 3.60 (m, 2H, 5′), 2.78 (m, 1H, isobutyryl CH), 2.49 (s, 3H, −8CH$_3$), 1.12 (d, 6H, J = 7.0 Hz, 2CH$_3$). ESMS, *m*/*e* calcd for $C_{15}H_{21}N_5O_6$ (M + H) 367.4, found 368.0.

8-Methyl-*N*^{2}-isobutyryl-5′-*O*-(dimethoxytrityl)guanosine (11). To 0.5 g (2.4 mmol) of the 8-methyl-*N* ^{2}-isobutyrylguanosine dried three times by evaporation of pyridine (15 mL) and suspended in 15 mL of

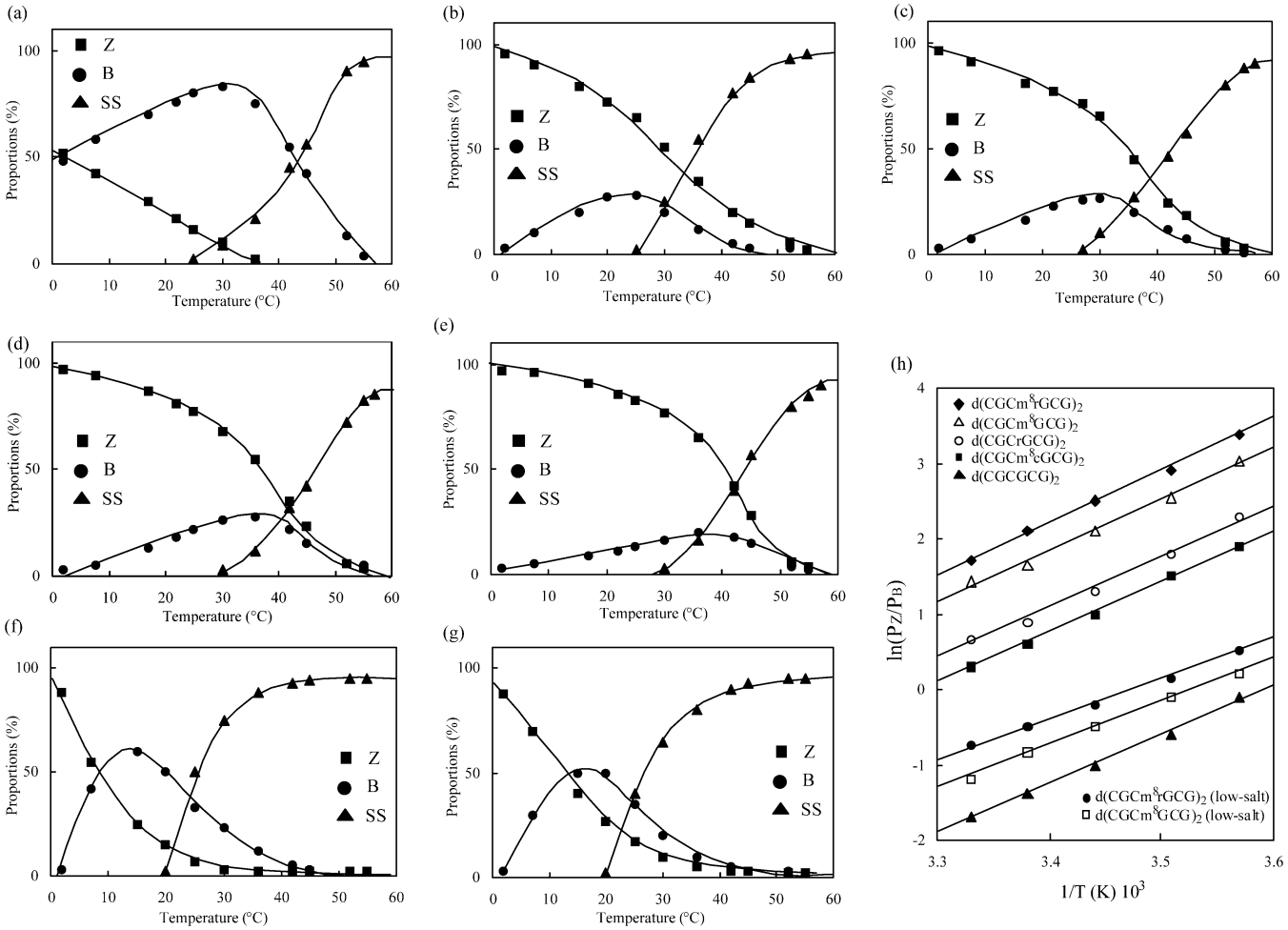


Figure 2. Proportions of Z, B, and SS conformations of (a) d(CGCGCG)$_2$, (b) d(CGCm^{8}cGCG)$_2$, (c) d(CGCrGCG)$_2$, (d) d(CGC^{8m}GCG)$_2$, and (e) d(CGC^{8m}rGCG)$_2$. Sample solutions contained 0.15 mM hexanucleotide (base concentration) in 3.0 M NaCl, 5 mM Na cacodylate buffer, pH 7.0. (f) and (g) are d(CGC^{8m}GCG)$_2$ and d(CGC^{8m}rGCG)$_2$ in the physiological salt concentrations (10 mM NaCl, 100 mM KCl). (h) van't Hoff plot for the B−Z conformational transition of these hexamers.

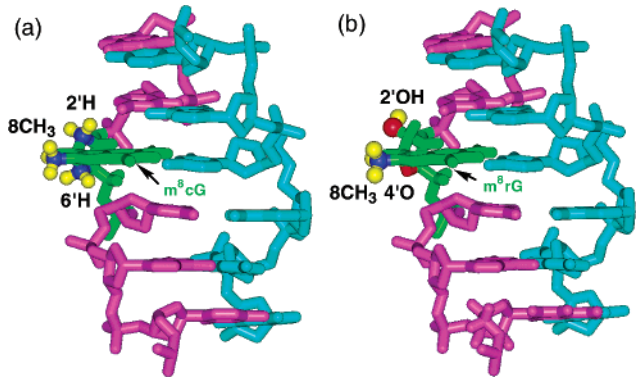


Figure 3. Molecular models of (a) d(CGCm^{8}cGCG)$_2$ and (b) d(CGCm^{8}rGCG)$_2$.

dry pyridine were added 0.7 g (3.7 mmol) of 4,4′-dimethoxytrityl chloride, 0.3 mL (2.1 mmol) of triethylamine, and 4.2 mg (0.04 mmol) of 4-dimethylaminopyridine. After it stood overnight, TLC (CH_2Cl_2:CH_3OH = 97:3) showed complete reaction. The reaction was cooled in an ice bath, and 50 mL of 5% $NaHCO_3$ was added. The mixture was extracted twice with 60 mL of CH_2Cl_2. The organic layer was evaporated to dryness, and the target compound was given by silica gel column chromatography: 0.5 g, yield was 61%. ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.16−7.29 (m, 9H, ph), 6.77 (m, 4H, ph), 5.82 (m, 1H, 1′), 4.78 (m, 1H, 2′), 4.27 (m, 1H, 3′), 3.98 (m, 1H, 4′), 3.71 (s, 6H, 2CH$_3$), 3.16 (m, 2H, 5′), 2.71 (m, 1H, isobutyryl CH), 2.45 (s, 3H, −8CH$_3$), 1.09 (d, 6H, J = 7.0 Hz, 2CH$_3$). ESMS, *m*/*e* calcd for $C_{36}H_{39}N_5O_8$ (M + H) 670.2, found 670.3.

8-Methyl-*N*^{2}-isobutyryl-5′-*O*-dimethoxytrityl-2′-*O*-*tert*-butyldimethylsilylguanosine (12). To a solution of **11** (1 g, 1.4 mmol) and imidazole (0.2 g, 3.0 mmol) in dry 1,2-dichloroethane (15 mL) was added *tert*-butyldimethylsilyl chloride (0.3 g, 2 mmol), and the mixture was kept at 20 °C for 16 h. A mixture of CH_2Cl_2 (20 mL) and 10% aqueous $NaHCO_3$ (15 mL) was added, and the organic layer was washed with water (15 mL), dried over Na_2SO_4, and evaporated in vacuo to dryness. The residue was purified by column chromatography on silica gel (hexane:EtOAc = 97:3) to give 0.3 g of target compound, yield was 31%. ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.19−7.38 (m, 9H, ph), 6.81 (m, 4H, ph), 5.85 (m, 1H, 1′), 4.94 (m, 1H, 2′), 4.35 (m, 1H, 3′), 4.08 (m, 1H, 4′), 3.80 (s, 6H, 2CH$_3$), 3.33 (m, 2H, 5′), 2.77 (m, 1H, isobutyryl CH), 2.47 (s, 3H, −8CH$_3$), 1.13 (d, 6H, J = 7.0 Hz, 2CH$_3$), 0.90 (s, 9H, ^{i}Bu), 0.07 (s, 3H, −CH$_3$), 0.01 (s, 3H, −CH$_3$). ESMS, *m*/*e* calcd for $C_{42}H_{53}N_5O_8Si$ (M + H) 784.0, found 784.1.

Preparation of the Amidite Monomers 7 and 13, and Solid-Phase Oligonucleotide Synthesis. The dimethoxytritylated guanine derivative **6** or **12** (0.5 mmol) was treated with dry *N*,*N*-diisopropylethylamine (1.5 mmol) and 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (0.75 mmol) in dry dichloromethane (5 mL) and stirred at room temperature for 24 h. The reaction was added to CH_2Cl_2 (50 mL), washed with 5% aqueous $NaHCO_3$, and dried over Na_2SO_4. Following column chromatography with *n*-hexane:acetone:triethylamine (49:49:2), an amount of **7** (0.7 mmol, 70%) or **13** (0.6 mmol, 84%) of

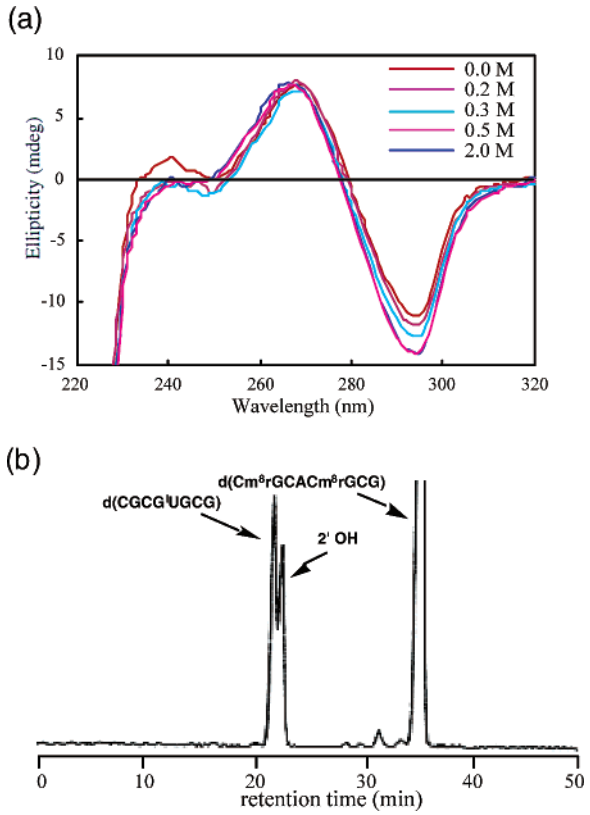


Figure 4. (a) CD spectra for d(CGCG^{I}UGCG)/d(Cm^{8}rGCACm^{8}rGCG) in 5 mM Na-cacodylate buffer, pH 7.0, at 10 °C at various NaCl concentrations. (b) HPLC profiles of UV-irradiated d(CGCG^{I}UGCG)/d(Cm^{8}rGCACm^{8}rGCG) in 50 mM NaI. The reaction mixture (100 μL) containing octamer (0.5 mM total base concentration) was irradiated for 2 h at 0 °C with a monochromator (302 nm). HPLC analysis was carried out on a Cosmosil 5C18-MS column; elution was with 0.05 M ammonium formate containing 3−8% acetonitrile, in a linear gradient at a flow rate of 1.0 mL/min for 50 min, at 30 °C. 2′OH: C2′α-hydroxylated product d(CGCrGUGCG).

Table 2. Midpoint NaCl Concentrations for B−Z Transitions of Various Deoxyoctanucleotides Derivatives[a]

	duplexes	NaCl
a	d(CGCG^{I}UGCG)/d(Cm^{8}GCACm^{8}GCG)	800
b	d(CGCrGUGCG)/d(Cm^{8}GCACm^{8}GCG)	420
c	d(CGCG^{I}UCGC)/d(Cm^{8}rGCACm^{8}rGCG)	0
d	d(CACATGCG)/d(Cm^{8}GCATm^{8}GTG)	2450
e	d(CACATGCG)/d(Cm^{8}rGCATm^{8}rGTG)	200
f	d(Cm^{8}GCACm^{8}GCG)/d(CGCGTG)	1100
g	d(Cm^{8}rGCACm^{8}rGCG)/d(CGCGTG)	210

[a] Experiments were carried out as described in the footnote to Table 1.

the amidite was isolated. ESMS for **13**, *m*/*e* calcd for $C_{51}H_{70}N_7O_9PSi$ (M + H) 984.2, found 985.1; ESMS for **7**, *m*/*e* calcd for $C_{46}H_{58}N_7O_7$ (M + H) 852.0, found 852.1. The corresponding 2-cyanoethyl phosphoramidite **7** or **13** was used at 0.12 M in acetonitrile solution, and coupling was allowed to proceed for 3 min, resulting in highly efficient coupling (>95%). Oligonucleotides, (**I**) d(CGCm^{8}cGCG), (**II**) d(CGCcGCG),[13] (**III**) d(CGCm^{8}rGCG), (**IV**) d(Cm^{8}rGCACm^{8}rGCG), and (**V**) d(Cm^{8}rGCATm^{8}rGTG), were synthesized. Methylamine/NH_4OH (28% in H_2O) (1:1) was used as a fast cleavage and deprotection reagent, with complete cleavage of modified oligonucleotides from the solid support occurring in 10 min at room temperature, and complete deprotection occurring in 10 min at 65 °C. Removal of the TBDMS-protecting group was done with 1 M TBAF in THF solution at room temperature. The reaction was quenched by addition of 0.4 mL of 1 M TEAA solution and was desalted on an ion exchange cartridge (Poly-Pak II).[14] The oligonucleotides were purified by reverse phase HPLC. ESI-MASS (negative mode), (**I**): calculated, 1804.3; found, 1804.4. (**II**): calculated, 1790.2; found, 1790.3. (**III**): calculated, 1822.0; found, 1822.1. (**IV**): calculated, 2454.5; found, 2454.7. (**V**): calculated, 2484.5; found, 2484.6. The guanosine (rG)-containing oligomer d(CGCrGCG) was purchased from JBioS. Concentrations and compositions of oligomers were determined by complete digestion of oligomers to mononucleosides using P1 nuclease and bacterial alkaline phosphatase.

CD Measurement and Thermodynamic Parameters Determination. CD spectra were measured with an AVIV model 62 DS/202 CD spectrophotometer. CD spectra of oligonucleotide solutions (0.15 mM base concentration in 5 mM sodium cacodylate buffer, pH 7.0, at 10 °C at various NaCl concentrations) were recorded using a 1 cm pathlength cell (Figure 1). The proportions of Z, B, and SS for these oligomers at 3 M NaCl as a function of temperature are shown in Figure 2, as previously reported.[9,15] To obtain the population of B- and Z-DNA (P_B and P_Z), we analyzed the CD spectra taking into account the three forms (B, Z, SS) present in solution in the temperature range covered (0−60 °C). Hence, at each temperature, we assumed that[15]

$$\Delta\epsilon^{295} = \Delta\epsilon_B{}^{295}P_B + \Delta\epsilon_Z{}^{295}P_Z + \Delta\epsilon_{SS}{}^{295}P_{SS} \quad (1)$$

$$1 = P_B + P_Z + P_{SS} \quad (2)$$

with the *P*'s being the molar fraction of the B, Z, and single strand components. The $\Delta\epsilon$'s, relative to the limit forms, have been estimated from the CD signal at 295 nm. They have been considered independent of the temperature and salt concentration, even though a small, but not insignificant, dependence was observed. P_{SS} at each temperature was determined by a subsequent UV melting experiment (of the same solution) monitoring the absorbance at the B−Z isosbestic point (270 nm).[15] Therefore, eqs 1 and 2, relative to each CD spectrum at a given temperature, were solved to provide estimates of P_B and P_Z. The B−Z conformational transition was analyzed by using the data collected below 25 °C. Thermodynamic parameters were calculated by plotting $\ln(P_Z/P_B)$ versus $1/T$: $\ln(P_Z/P_B) = (\Delta H°/R)(1000/T) - \Delta S°/R$ in 3.0 M NaCl or in 10 mM NaCl and 100 mM KCl, 5 mM sodium cacodylate buffer, pH 7.0. The free energy change associated with the duplex formation at 297 K, $\Delta G_{297K}°$, was calculated from the obtained $\Delta H°$ and $\Delta S°$ values using the following equation: $\Delta G_{297K}° = \Delta H° - T\Delta S°$.

Supporting Information Available: Information on the NOESY spectra of 8-methyl-2′-deoxycarbaguanosine (m^{8}cG) and 8-methyl-2′-deoxyguanosine (m^{8}G) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA036233I

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