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The Spermine–Bisaryl Conjugate as a Potent Inducer of B- to Z-DNA Transition

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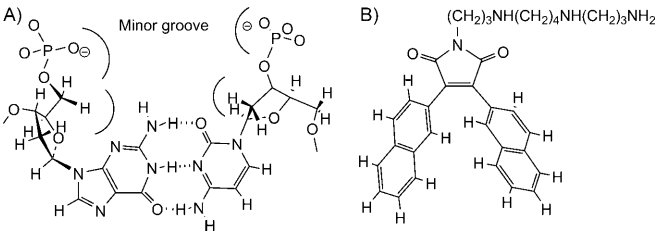
Abstract: DNA containing alternating purine and pyrimidine repeats has the potential to adopt the Z-DNA structure, one of the well-studied structures besides A- and B-DNA. Despite a number of molecular models that have been proposed to explain the mechanism for B→Z transition, there is continued discussion on the mechanism and physiological role of this transition. In this study, we have found that the bis(2-naphthyl)-maleimide–spermine conjugate (**3c**) exhibits a remarkable ability to cause the B→Z transition of $d(CGCGCG)_2$ at low salt concentrations. Using isothermal titration calorimetry (ITC) we show that the B→Z transition induced by **3c** is both enthalpically and entropically favorable. The ligand might effect the dehydration of B-DNA, which leads to the B→Z transition. Interestingly, an intermediate CD between the B and Z forms was observed in the pH-dependent transition in the presence of the ligand. The unique structure and characteristics of the ligand designed in this investigation will be useful for the study of Z-DNA.

Keywords: B–Z transition · circular dichroism · DNA · isothermal titration calorimetry · surface plasmon resonance

Introduction

DNA containing alternating purine and pyrimidine repeats has the potential to adopt the Z structure, one of the well-studied structures besides A- and B-DNA. Z-DNA is a left-handed helix in which the purines are in the *syn* conformation and the pyrimidines in the *anti* conformation.[1] It has been shown that Z-DNA exists in vivo under physiological conditions as a transient structure occasionally induced by a biological process, such as transcription, the methylation of cytosine, and the level of DNA supercoiling.[2,3] Several proteins have been identified that bind to Z-DNA.[2] Recently, the B→Z transition has been utilized as a key event in nanodevices that are responsive to an external signal.[4–6] The B→Z transition is observed under a variety of conditions including high salt concentrations (4 M NaCl), in the presence of transition metal complexes[7] ($[Co(NH_3)_6]^{3+}$), or small molecules, such as polycations (spermine), chiral binding molecules, etc.[8,9] The B→Z transition is cooperative, and the length of the polymer is important for the process, for instance, the B form of poly[d(G-C)·d(C-G)] is more easily transformed into the Z form with increasing polymer length.[10,11] Despite a number of molecular models that have been proposed for the mechanism and the physiological role of the B→Z transition, there is continued discussion. We now report that spermine-conjugated bisaryl ligands (Scheme 1) effectively induce the B→Z transition of $d(CGCGCG)_2$ at low salt concentrations.



Scheme 1. Structural characteristics of the topology of: A) Z-DNA, and B) a representative ligand (**3c**) synthesized in this study. Because of the *syn* conformation of guanosine, the minor groove of Z-DNA is narrower and the phosphate anions are closer than those of B-DNA.

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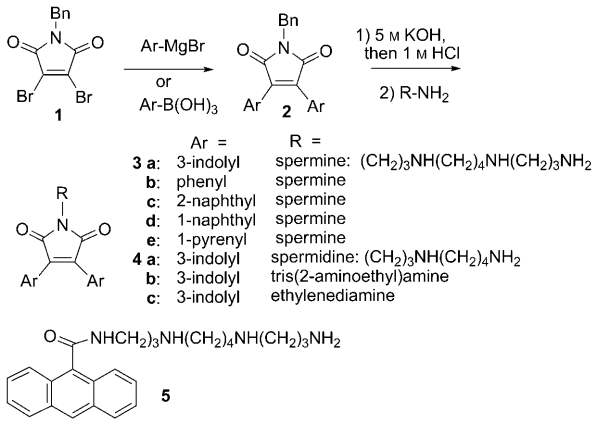
Results and Discussion

In the unique topology of Z-DNA, the guanine bases in the *syn* conformation base pair with the cytosine bases in the *anti* conformation, and the minor groove is narrower and deeper than that of B-DNA.[1] It has been reported that in a low temperature crystal structure of the spermine complexed with Z-DNA duplex, the spermine in the minor groove decreases the cross-groove electrostatic repulsions between the phosphate anions within the Z-DNA.[12] The crystal structure has been reported to bind two molecules of tetra-amine, $NH_2((CH_2)_3NH)_3H$, in the minor groove of Z-$d(CGCGCG)_2$ formed at high $MgCl_2$ concentrations.[13] During the initial stage of this study, we hypothesized that spermine conjugated with aromatic groups would produce electrostatic interactions between the cations of spermine and phosphate anions within the minor groove of Z-DNA. Based on the crystal structure in which the spermine is located in the minor groove of Z-DNA, it was also expected that the aromatic groups would provide additional stacking interactions. Because the space between the G–C base pair of Z-DNA is narrower than that of B-DNA, the bisaryl structure, as shown in Scheme 1 B, was postulated to be a better candidate for a good fit for such a space in the Z-DNA.

The structure of the designed compounds and their syntheses are summarized in Scheme 2. The dibromomaleimide derivative (**1**) was coupled to the aromatic compounds, and the resulting bisaryl derivatives (**2**) were transformed into the corresponding anhydrides. Aminolysis of the anhydride with the amine was followed by acid-catalyzed imide closure to produce the bisaryl-maleinimide-amine derivatives (**3** and **4**). They were used as the HCl or TFA salts. The spermine–anthracene conjugate (**5**) has been reported to bind poly[d(G-C)·d(C-G)] by anthracene intercalation,[14] and was used as a reference compound.

The B→Z transition was analyzed by CD spectrum changes by using $d(CGCGCG)_2$ as the B-DNA substrate. Examples with **3a**, **3c** and **3d** are shown in Figure 1 A, B and C, respectively. Addition of the bisaryl ligand decreased the intensity of the positive (at about 278 nm) and negative bands (at about 253 nm) that are characteristic of B-DNA; this was accompanied by an increase in intensity of the negative band at about 296 nm and the positive band at about 270 nm, and clearly indicates B→Z transition. The B→Z transition reached equilibrium immediately after the addition of the bisaryl ligand. The concentrations of ligand needed to induce a 50% B→Z transition (EC_{50}) are summarized in Table 1. As clearly shown by the EC_{50} values, the aromatic group is a key determinant for the B→Z transition ability.



Scheme 2. Synthesis of bisaryl ligands and the structure of the reference compound **5**.

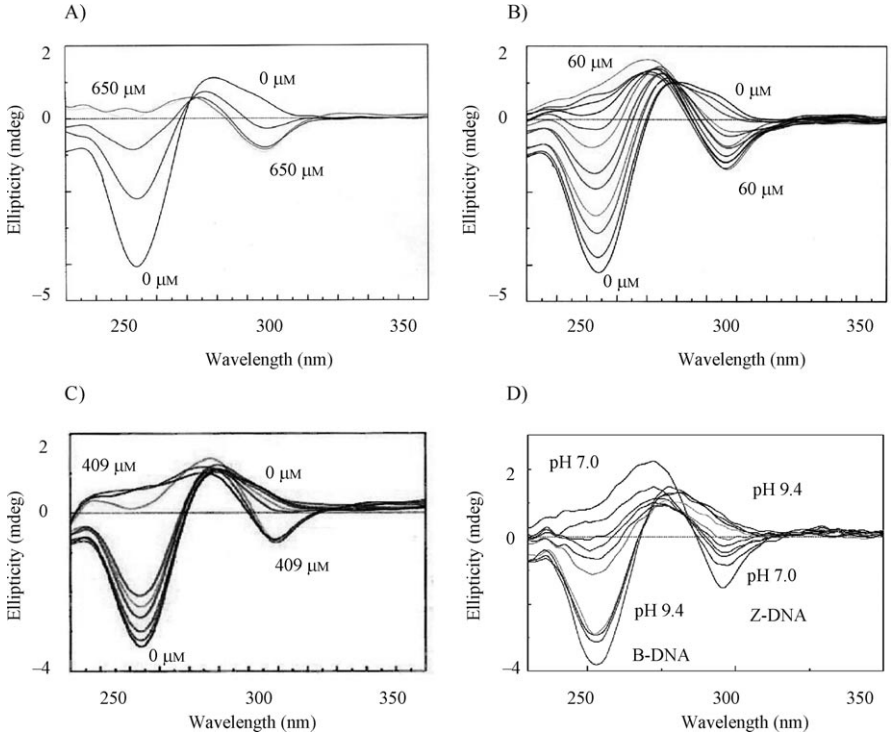


Figure 1. CD change in B-DNA after the addition of the bisaryl ligands. A) B→Z transition induced by **3a**, B) B→Z transition induced by **3c**, C) B→Z transition induced by **3d**, D) pH-dependent B→Z transition. A), B), C) The CD spectrum was measured by using B-DNA $d(CGCGCG)_2$ (20 μM) in Na cacodylate buffer (5 mM) containing NaCl (100 mM) at pH 7.0. Ligand concentrations: A) 0 to 650 μM, B) 0 to 60 μM, C) 0 to 409 μM, D) a mixture containing 20 μM $d(CGCGCG)_2$ and 60 μM **3c** in Na cacodylate (5 mM) and NaCl (100 mM) was used. The pH was raised from 7 to 9.4 by the addition of NaOH (0.1 M).

Table 1. Properties of the bisaryl ligands **3** and **4**.

Ligand	EC_{50} [μM][a]	ΔT_m [°C] Z-DNA[b]	K_a [10^6 M^{-1}][c] Z-DNA	B-DNA
3a	250	7.0	0.28	1.1
3b	>600	3.8	–	–
3c	18	18	3.2	0.7
3d	88	6.0	0.04	0.26
3e	29	_[e]	_[e]	_[e]
4a	370	–	–	–
4b	>600	1.3	–	–
4c	>600	0.5	–	–
spermine	no[d]	5.1	0.12	0.24
5	no[d]	–	0.11	0.052
(−)-daunorubicin	–	–	0.01[f]	0.012[f]
helicene (*P*)-A	–	–	0.08[g]	0.014[g]

[a] The EC_{50} values for the B→Z transition were obtained from the CD experiments. [b] Melting temperatures were measured by using d-$(CGCm^8GCG)_2$ (5 μM) and ligand (20 μM) in Na cacodylate buffer (5 mM) containing NaCl (100 mM) at pH 7.0. [c] The binding constants were obtained by SPR measurements by using sensor chips immobilized with 5′-biotin-conjugated d(TTTTCGCGCG-TTTT-CGCGCG) as the B-DNA and 5′-biotin-conjugated d(TTTTCGCm^{8}GCG-TTTT-CGCm^{8}GCG) as the Z-DNA. [d] No CD change was observed at 600 μM. [e] Measurements were not done because of the overlapping UV absorption of **3e**. [f] Data taken from ref. [8]. [g] Data taken from ref. [9].

The effect for the B→Z transition is in the order **3c**>**3e**>**3d**>**3a**>**3b** among the spermine conjugates. The anthracene–spermine conjugate (**5**), a potent B-DNA binder,[14] did not induce B→Z transition (Figure S9A and B in the Supporting Information). The bis(2-naphthyl) derivative (**3c**) was found to have the highest efficacy among the ligands tested in this study.

The amino group also has a significant effect on the efficiency of B→Z transition, and spermine produced the best effect compared to spermidine (**4a**), tris(2-aminoethyl)-amine (**4b**) or ethylene diamine (**4c**; Figure S8 in the Supporting Information). Neither spermine nor bis(3-indolyl)-maleimide induced the B→Z transition of $d(CGCGCG)_2$ B-DNA at the low salt concentrations used in this study (Figure S6 in the Supporting Information). The bisaryl–spermine ligands are unique examples of small molecules demonstrating a high efficiency in inducing B→Z transition. The Z form returned to the B form when the pH was raised from 7.0 to 9.4 (Figure 1D), indicating the importance of the electrostatic binding between the ammonium cations and the phosphate anions for the B→Z transition. Interestingly, Figure 1D shows two isodichroic points at around 283 and 267 nm, and the existence of an intermediate CD is suggested in addition to the B and Z forms. The intermediate CD resembles that of the B form. The Z form changed to the intermediate form when the pH was raised from 7.0 to about 8.1, and the intermediate form returned to the B form at higher pH. From the pK_a values of the carbamate conjugate of spermine (10.1, 8.6, 7.3),[15] the bisaryl ligand **3c** with the decreased positive charge should bind the intermediate complex in the B→Z transition. This intermediate CD is similar to that observed during the slow B→Z transition of poly[d-(G-C)·d(C-G)] induced by the cationic comb-type copolymer.[16]

To determine the role of the spermine-conjugated bisaryl ligand in the B→Z transition, the binding properties of the ligand were investigated. Because 8-methyl-2′-deoxyguanosine (m^8G) is known to stabilize the Z form when it is incorporated into a CG repeat sequence, $d(CGCm^8GCG)_2$ was used as the standard Z-DNA.[17] The effect of the ligand on the thermal stability of the Z-DNA was estimated by measuring the UV melting temperature (T_m); the increase in the T_m values are summarized in Table 1. The ligand **3c** showed a high stabilizing effect with Z-DNA. However, because there is not a certain relationship between the ED_{50} and ΔT_m values, the increase in the thermal stability of the Z-DNA in the presence of ligand is not thought to be the cause of the B→Z transition.

Next, we investigated the binding properties of the ligand with SPR measurements. The 5′-biotin-conjugated d(TTTTCGCGCG-TTTT-CGCGCG) sequence was immobilized on a sensor chip for measurement of ligand binding.[18] This sequence formed the B-type duplex with a loop structure, but was not transformed to the Z form after addition of **3c** under low salt conditions (Figure S9C in the Supporting Information). However, 5′-biotin-conjugated d(TTTTCGCm^{8}GCG-TTTT-CGCm^{8}GCG) formed the Z-DNA under the same conditions (Figure S9D in the Supporting Information). Accordingly, the binding properties for the B- and Z-DNA were independently measured by SPR with these duplexes. Examples of the SPR results with **3c** and B- and Z-DNA are shown in Figure 2A and B, respectively. The SPR data (Figure S10 in the Supporting In-

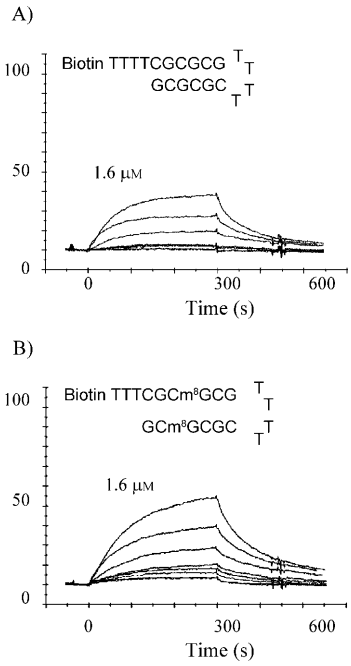


Figure 2. SPR sensorgrams obtained by using an ODN with a loop structure in the B and Z forms. A) Transition to B-DNA, B) transition to Z-DNA. The biotin-conjugated ODN shown in the graph was immobilized on an avidin-coated sensor chip and **3c** (0.5–1.6 μM) was analyzed for 5 min.

formation) were analyzed by using the standard method installed on the instrument to give the equilibrium association constants (K_a), which are summarized in Table 1. Among the tested ligands, only **3c** showed a higher binding affinity for the Z-DNA than B-DNA. In this case, the bisaryl-maleimide part increased the binding affinity to Z-DNA by 20–30 times compared to spermine; this suggests an important role for the geometry of the ligand in DNA binding. Although **3a** and **3d** induced B→Z transition with moderate potency, their binding affinities to Z-DNA were relatively low when compared to B-DNA. Accordingly, the binding affinity of the ligand to Z-DNA could not be correlated with the potency for the B→Z transition.

To obtain further insights into the origin of the potency of **3c** to induce B→Z transition, thermodynamic parameters were obtained by ITC. Ligand was added into a solution containing $d(CGCGCG)_2$ in the B form or $d(CGCm^8GCG)_2$ in the Z form. Because **3c** induces the B→Z transition of $d(CGCGCG)_2$ under the titration conditions, the parameters obtained by using the B form include those for the B→Z transition. Figure 3 shows the heats obtained from the titration of **3c** into B- or Z-DNA. The isotherm shown at the bottom of Figure 3A suggests that the initial step with less than 10 μM is different to when higher concentrations of ligand are used, as highlighted by the dotted square. Such a change in the isotherm was confirmed by a titration with the ligand at low concentrations (Figure 3A, inset). The thermodynamic parameters obtained for **3c** are shown in Table 2 together with the reported ones. The thermodynamic parameters obtained with **3c** and B-DNA clearly indicate that the initial binding step is entropy driven, and are similar to those obtained with spermine in that the binding of spermine to $d(C\text{-}G)_{20}$, which is not B→Z inducible, occurs with a small positive ΔH and a larger $-T\Delta S$ (Table 2).[19] It is likely that the initial binding occurs with the spermine part of **3c**. However, the second step involving the binding and B→Z transition was shown to be both enthalpically as well as entropically favorable. This is a characteristic feature of B→Z transition induced by **3c** compared to the well-known B→Z transition induced by $[Co(NH_3)_6]^{3+}$ or Na^+, which are entropy-driven processes[20] (Table 2). The binding stoichiometry defined as the molar ratio was 1.07 below 10 μM and changed to 3.5 at higher concentrations. The isotherm obtained with Z-DNA indicated one-phase binding and is favorable both entropically and enthalpically. The stoichiometry was shown to be Z-DNA/ligand **3c** = 1:4.0.

Table 2. Thermodynamic parameters obtained by the titration of **3c** against B- and Z-DNA.[a]

Inducer	K_a [10^6 M^{-1}]	H [kJ mol^{-1}]	G [kJ mol^{-1}]	$-TS$ [kJ mol^{-1}]	N[d]
B–**3c** < 10 μM	1.23	3.34	−77.23	−80.57	1.07
B–**3c** > 10 μM	0.62	−21.77	−76.05	−54.28	3.5
Z–**3c**	3.08	−32.56	−72.23	−39.58	4.06
spermine[b]	0.0345	1.85	−25.72	−27.57	6.58
Na^+[c]	0.0002	2.93	−11.72	−15.03	5.3
$[Co(NH_3)_6]^{3+}$[c]	0.1	3.01	−18.00	−20.93	5.1

[a] Thermodynamic parameters were obtained with the curve-fitting method by using standard procedures, two independent models for titration with the B-DNA and one independent model for titration with the Z-DNA. [b] The titration of spermine was done against $d(GC)_{20}$ and did not induce B→Z transition.[19] [c] The titration was done against $(dm^5C\text{-}dC)_4$ and induced B→Z transition; the parameters are reported per base-pair.[20] [d] The binding stoichiometry is defined as molar ratio [ligand]/[duplex].

The complex structure formed with **3a** and $d(CGCm^8GCG)_2$ in the Z form was investigated by ^{1}H NMR spectroscopy. The ligand **3a** was used because of its high solubility in the buffer for the ^{1}H NMR measurements. The NMR spectra of free **3a**, free Z-DNA, and the complex are compared in Figure 4. All protons of the indolyl groups were shifted upfield (Figure 4, A vs. B). Some signals of the Z-DNA (8-H of G6, and 6-H of C)[15] also displayed upfield shifts by the formed complex (Figure 4, B vs. C), suggesting that the indolyl groups were stacked with the G–C base pairs at the terminal position. Thus, the complex can be postulated to have this structure as the bisaryl parts come close to the G–C pairs at the terminal position and the cationic spermine interacts with the phosphate anions in the minor groove and/or at the exterior of the duplex.

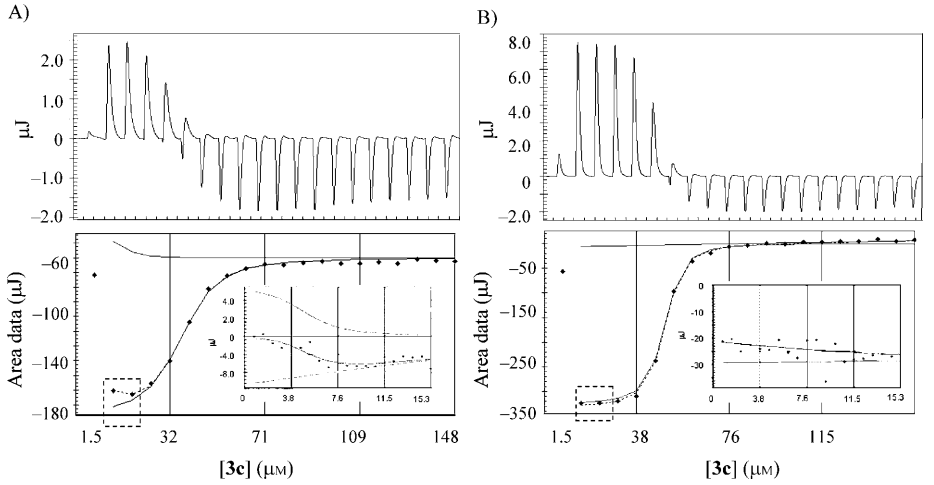


Figure 3. Unsubtracted calorimetric binding heats obtained from titration of ligand **3c** against B-DNA $d(CGCGCG)_2$ (A) and Z-DNA $d(CGC^mGCG)_2$ (B). The positive (endothermic) heats show the ligand–DNA binding, and the negative (exothermic) heats represent the ligand heat of dilution. Subtraction of the heats of dilution obtained from the separate titrations from the complete titration curve yielded the binding isotherm reported at the bottom. The ligand solution was added to the solution containing 10 μM B-DNA or Z-DNA in Na cacodylate buffer (5 mM) containing NaCl (100 mM) at pH 7.0. The ligand concentration ranged from 0 to 148 μM. The inset shows the isotherm obtained when the ligand concentration ranged from 0–15.3 μM.

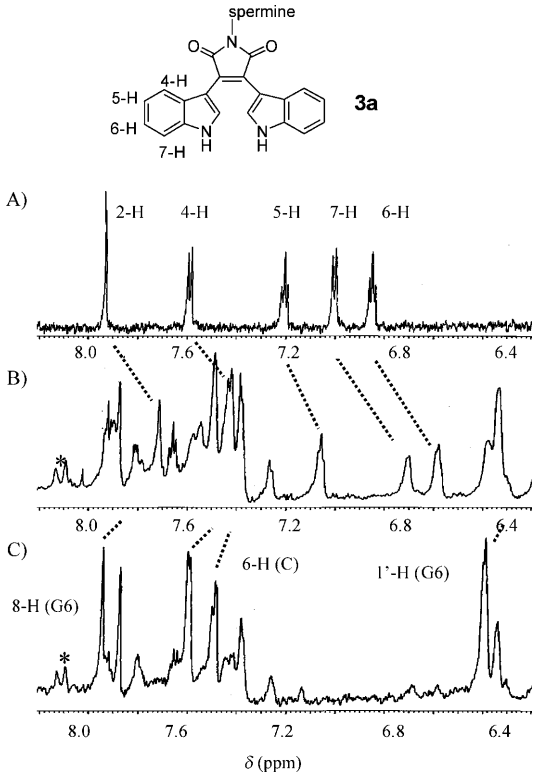


Figure 4. ^{1}H NMR spectra of the complex of **3a** with Z-DNA. A) Ligand **3a** only, B) the complex of **3a** and Z-DNA, C) Z-DNA only. The marked peaks correspond to the coexisting B-DNA.[17] The ^{1}H NMR spectra was obtained by using 120 μM Z-DNA and 100 μM **3a** in D_2O containing Na cacodylate (5 mM), NaCl (100 mM) at pH 7 and 10 °C.

Discussion

The B→Z transition is associated with nucleotide dehydration.[21–23] It has been also reported that neutral solutes facilitate the B→Z transition through osmotic stress.[24,25] The thermodynamic parameters of **3c** obtained by ITC have suggested that an entropy-driven initial binding of the spermine part is followed by an entropically and enthalpically favorable process. The NMR spectroscopy study has shown that the aromatic groups assemble around the terminal G–C pairs. Thus, in the second step, which is an enthalpically and entropically favorable process, association of the hydrophobic bisaryl groups might cause dehydration of the B-DNA, and enhance electrostatic binding of the spermine part to the phosphate backbone of the DNA, eventually leading to the B→Z transition. The geometry of the bisaryl group is also a significant determinant for the potency in the B→Z transition. Taking into account that some intercalators inhibit B→Z transition,[26–29] it should be noted that the 2-naphthyl groups of **3c** are twisted towards each other and are not likely to intercalate between the base pairs. The role of the bisaryl geometry for the B→Z transition will be further studied in detail. It is also notable that the intermediate CD induced by **3c** in the pH-dependent B→Z transition (Figure 1 D) was stable under the conditions used in this study. Such a stable intermediate form might allow structure determination, which will be useful to gain insight into the molecular mechanism underlying the B→Z transition.

Conclusion

In conclusion, we have found that the bis(2-naphthyl)maleimide–spermine conjugate (**3c**) exhibits a remarkable ability for the induction of the B→Z transition of $d(CGCGCG)_2$ at low salt concentrations. The ITC study has revealed that the B→Z transition induced by **3c** is both enthalpically and entropically favorable. The ligand may effect the dehydration of B-DNA that leads to the B→Z transition. Interestingly, an intermediate CD between the B and Z forms was observed in the pH-dependent transition in the presence of the ligand. The unique structure and characteristics of the ligand designed here will be useful for the study of Z-DNA.

Experimental Section

DNA oligomers: DNA oligomers were synthesized with an automated DNA synthesizer by using conventional amidite chemistry. The DMTr-protected DNA was cleaved from the resin with NH_4OH solution and purified by HPLC equipped with a ODS column by using TEAA·CH_3CN (0.1 M) linear gradient system. The DMTr protecting group of the purified oligonucleotide (ODN) was cleaved in aqueous AcOH (10%) and the mixture was washed with ether. The purity of the ODN was confirmed by MADI-TOF MS and UV measurements. The concentration of the stock solution was estimated by UV absorbance at 260 nm, which was further confirmed by HPLC analysis of the component nucleosides after enzymatic hydrolysis with bacterial alkaline phosphatase (BAP) and snake venom phosphodiesterase (VPDE), with less than 5% error.

CD measurements: CD spectra were recorded by using a Jasco-J720 spectrometer with a 2 mm quartz glass cell (200 μL) at the constant temperature of 20 °C. A solution of $d(CGCGCG)_2$ B-DNA (20 μM) or $d(CGCm^8GCG)_2$ Z-DNA (20 μM) in buffer (5 mM Na cacodylate containing 100 mM NaCl at pH 7) was used for the CD measurements. The ODN solution was titrated with the ligand stock solution (1 μL each). All CD data were blank corrected, and are reported as the average of quadruplicate scans.

Measurement of UV melting temperature: In the UV melting experiments, a solution of Z-DNA (5 μM in Na cacodylate buffer 5 mM containing 100 mM NaCl, pH 7) was used. The temperature was raised or lowered at the rate of 1 °C min^{-1} between 10 and 60 °C.

Surface plasmon resonance (SPR) measurements: 5′-Biotin-conjugated d(TTTTCGCGCG-TTTT-CGCGCG) or d(TTTTCGC-m^{8}GCG-TTTT-CGC-m^{8}GCG) (10–500 nM) in HEPES (10 mM) and NaCl (0.15 M) at pH 7.4 was immobilized on a sensor chip SA at 20 °C. One flow channel was always left as a blank for reference. The SPR measurements were performed at 25 °C by using Na cacodylate buffer (5 mM) containing NaCl (100 mM) at pH 7.0 as the running buffer. Binding was measured at 20 μL min^{-1} for 120 s and dissociation for 150 s. A Biacore 3000 (GE Healthcare) was used for the SPR measurements.

Isothermal titration calorimetry (ITC): The ligand solution (**3c**; 1 mM) was added to the final dialysate obtained from ODN dialysis, and was titrated into 10.0 μM $d(CGCGCG)_2$ or $d(CGCm^8GCG)_2$ in Na cacodylate buffer (5 mM) containing NaCl (100 mM) at pH 7.0 and 25.0 °C. The first injection was 2 μL, and then 10 μL of each ligand stock was added to the ODN solution. The data obtained with the first injection were omitted from the analysis. The ODN concentrations are reported as molar concentrations of double strands. All samples were degassed prior to use by

application of vacuum and simultaneously stirred on a magnetic plate. A NanoITC SV (TA Instruments) was used for the calorimetric measurements.

^{1}H NMR measurements: ^{1}H NMR spectra were recorded by using a Varian UNITY INOVA 600 MHz spectrometer with **3a** (100 μM) and d-$(CGCm^8GCG)_2$ (120 μM) in D_2O containing Na cacodylate (5 mM) and NaCl (100 mM) at 10 °C. Each ^{1}H NMR spectra of **3a** or d$(CGCm^8GCG)_2$ was recorded under the same conditions.

The synthesis of 3c as a general procedure: A solution of CsF (0.53 g, 3.48 mmol, in 1.5 mL H_2O) was added to a mixture of 1-benzyl-3,4-dibromopyrole-2,5-dione (0.30 g, 0.87 mmol), 2-naphthalene boronic acid (0.45 g, 2.61 mmol), $Pd(PPh_3)_2Cl_2$ (30.50 mg, 0.0435 mmol) and $PhCH_2NEt_3Cl$ (10 mg, 0.0435 mmol) in degassed toluene–H_2O (3.6 mL/1.8 mL), and was heated at 60 °C for 30 h. The reaction mixture was diluted with AcOEt (15 mL), and washed successively with HCl (1 M; 15 mL) and brine. The organic layers were dried over Na_2SO_4, and evaporated to give a crude residue, which was purified by column chromatography (silica gel, hexane/AcOEt, 7:1) to give 1-benzyl-3,4-bis(2-naphthyl)-pyrrole-2,5-dione (0.38 g, 99 %) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=4.86$ (2H, s), 7.27–7.37 (5H, m), 7.46–7.53 (6H, m), 7.67 (2H, d, $J=8.6$ Hz), 7.77 (2H, d, $J=7.9$ Hz), 7.81 (2H, d, $J=7.9$ Hz), 8.21 ppm (2H, s); IR: $\tilde{\nu}=3675$, 2973, 1766,1702, 1401 cm^{-1}; MS (ESI): m/z: $[M+H]^+$: 440.10.

The above compound (0.15 g, 0.35 mmol) was dissolved in 5 M NaOH (2.2 mL)–ethanol (9.1 mL), and the solution was stirred for 21 h at room temperature. The reaction mixture was acidified with HCl (1 M; 12 mL) and extracted with $CHCl_3$. The organic layers were dried over Na_2SO_4, and evaporated to give a crude residue, which was purified by column chromatography (silica gel, toluene) to give 3,4-bis(2-naphthyl)furan-2,5-dione as a yellow solid (0.113 g, 93 %). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=$ 7.42 (2H, d, $J=8.6$), 7.51–7.59 (4H, m), 7.57 (2H, dd, $J=7.3$ Hz,), 7.74 (2H, d, $J=8.6$ Hz), 7.82 (2H, d, $J=7.9$ Hz), 7.86 (2H, d, $J=8.2$ Hz), 8.29 ppm (2H, s); IR: $\tilde{\nu}=1839$, 1762, 865, 822, 738 cm^{-1}; MS (ESI): m/z: $[M+H]^+$: 351.21.

A solution of the above compound (16.6 mg, 0.051 mmol, in 0.5 mL ether) was added slowly into a solution of spermine (22 mg, 0.152 mmol, in 3 mL methanol), and the mixture was stirred for 1 h at room temperature. The precipitates were collected and dissolved in toluene (0.75 mL). The solution was heated under reflux for 19 h, and the solvents were removed to give a residue, which was purified by column chromatography (amino-silica gel, $CHCl_3$/MeOH, 100:0 to 5:1) to give **3c** as a red solid. This material was transformed to the corresponding hydrochloride with $HCl{\cdot}CH_3OH$ (36 mg, 15 %).

The ligand **3c** was further purified by HPLC (ODS column, 0.05 % TFA in CH_3CN/0.05 % TFA in H_2O, 40:60 for 20 min, then 100:0) for measurements of binding properties. The concentration of the stock solution of **3c** was determined by comparing the integration values of the ^{1}H NMR signals with those of maleimide as the internal standard. ^{1}H NMR (400 MHz, CD_3OD): $\delta=1.80$ (4H, br), 2.05 (2H, m), 2.12 (2H, m), 2.99–3.20 (10H, m), 3.86 (2H, t, $J=6.4$ Hz), 7.04 (2H, d, $J=8.5$ Hz), 7.39 (2H, d, $J=7.6$ Hz), 7.45–7.49 (4H, m), 7.59 (2H, d, $J=7.6$ Hz), 7.67 (2H, br), 7.78 ppm (2H, s); ^{13}C NMR (125 MHz, CD_3OD): $\delta=172.3$, 137.6, 135.1, 134.4, 131.7, 129.7, 129.0, 128.8, 128.6, 127.7, 127.5, 48.2, 48.2, 46.8, 45.8, 37.8, 36.2, 26.9, 25.3, 24.2, 24.2 ppm; HRMS (ESI): m/z: calcd for $C_{34}H_{39}N_4O_2$ $[M+H]^+$: 535.3068; found 535.3066.

Compound 3a: ^{1}H NMR (400 MHz, CD_3OD): $\delta=1.83$ (4H, brs), 2.11 (4H, m), 3.07–3.12 (10H, m), 3.79 (2H, t, $J=6.4$ Hz), 6.60 (2H, dd, $J=$ 7.3, 7.9 Hz), 6.83 (2H, d, $J=7.9$ Hz), 6.97 (2H, dd, $J=7.3$, 7.9 Hz), 7.33 (2H, d, $J=7.9$ Hz), 7.75 ppm (2H, s); ^{13}C NMR (125 MHz, CD_3OD): $\delta=$ 174.1, 137.7, 130.1, 128.6, 127.0, 123.0, 122.5, 120.7, 112.4, 107.5, 48.2, 48.1, 46.8, 45.8, 37.8, 35.8, 27.0, 25.3, 24.2, 24.2 ppm; IR: $\tilde{\nu}=3385$, 2984, 2817, 2323, 1754, 1689, 1630, 1528, 1454, 1409, 1362 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{30}H_{37}N_6O_2$ $[M+H]^+$: 513.2973; found 513.2952.

Compound 3b: ^{1}H NMR (400 MHz, CD_3OD): $\delta=1.83$ (4H, brs), 2.11 (4H, brs), 3.03–3.12 (8H, m), 3.78 (2H, t, $J=6.4$), 7.34–7.44 ppm (10H, m); ^{13}C NMR (125 MHz, CD_3OD): $\delta=172.2$, 137.9, 131.0, 130.9, 130.1, 129.5, 48.2, 48.1, 46.7, 45.8, 37.8, 36.1, 26.8, 25.3, 24.2, 24.2 ppm; IR: $\tilde{\nu}=$ 3286, 3057, 2931, 2861, 1766, 1698, 1443, 1405 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{26}H_{35}N_4O_2$ $[M+H]^+$: 435.2755; found 435.2749.

Compound 3d: ^{1}H NMR (400 MHz, CD_3OD): $\delta=1.73$ (4H, brs), 1.96–2.04 (2H, m), 2.11–2.16 (2H, m), 2.99–3.08 (8H, m), 3.18 (2H, t, $J=$ 7.6 Hz), 3.84 (2H, t, $J=6.5$ Hz), 7.26 (2H, t, $J=7.8$ Hz), 7.37 (5H, brs), 7.61 (1H, br), 7.59 (2H, d, $J=7.6$ Hz), 7.77–7.84 ppm (4H, brs); ^{13}C NMR (125 MHz, CD_3OD): $\delta=172.3$, 141.7, 134.9, 132.0, 131.2, 129.6, 129.4, 128.6, 127.2, 127.1, 126.5, 126.0, 48.2, 46.8, 45.8, 37.8, 36.5, 26.9, 25.3, 24.2, 24.2 ppm; IR: $\tilde{\nu}=3045$, 2838, 1768, 1702, 1675, 1200, 1130 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{34}H_{39}N_4O_2$ $[M+H]^+$: 535.3068; found 535.3062.

Compound 3e: ^{1}H NMR (400 MHz, CD_3OD): $\delta=1.82$–1.84 (4H, brs), 2.00–2.07 (2H, m), 2.26–2.30 (2H, m), 3.00 (2H, t, $J=7.8$ Hz), 3.05–3.08 (4H, m), 4.03 (2H, t, $J=6.4$ Hz), 7.73 (2H, d, $J=9.2$ Hz), 7.89–8.11 ppm (16H, m); ^{13}C NMR (125 MHz, CD_3OD): $\delta=172.6$, 141.9, 133.6, 132.1, 131.6, 130.3, 129.6, 129.2, 128.7, 128.5, 128.3, 127.9, 127.1, 126.8, 126.6, 125.6, 125.4, 125.1, 48.9, 48.2, 46.9, 45.8, 37.8, 36.7, 27.1, 25.3, 24.3, 24.2 ppm; IR: $\tilde{\nu}=3033$, 2840, 1764, 1704, 1679 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{46}H_{42}N_4O_2$ $[M+H]^+$, 683.3381; found 683.3388.

Compound 4a: ^{1}H NMR (400 MHz, CD_3OD): $\delta=1.50$–1.59 (4H, m), 1.90 (2H, q, $J=6.9$ Hz), 2.59 (2H, t, $J=7.0$ Hz), 2.65 (4H, dt, $J=3.4$, 6.7 Hz), 3.72 (2H, d, $J=6.7$ Hz), 6.59 (2H, dt, $J=0.9$, 8.2 Hz), 6.83 (2H, d, $J=8.2$ Hz), 6.97 (2H, dt, $J=0.9$, 8.2 Hz), 7.32 (2H, d, $J=8.2$ Hz), 7.73 ppm (2H, s); ^{13}C NMR (125 MHz, CD_3OD): $\delta=174.1$, 137.8, 130.1, 128.6, 127.0, 123.1, 122.5, 120.7, 112.5, 107.5, 48.2, 46.8, 40.0, 35.8, 25.5, 24.2, 19.3 ppm; IR: $\tilde{\nu}=3373$, 2928, 2857, 2345, 1753, 1688, 1614, 1529, 1436 cm^{-1}; MS (ESI): m/z: calcd for $C_{27}H_{29}N_5O_2$ $[M+H]^+$: 456.2394; found 456.2390.

Compound 4b: ^{1}H NMR (400 MHz, CD_3OD): $\delta=2.96$ (6H, t, $J=$ 5.2 Hz), 3.11 (4H, t, $J=6.7$ Hz), 3.81 (2H, t, $J=5.8$ Hz), 6.61 (2H, dd, $J=$ 7.3, 7.7 Hz), 6.85 (2H, d, $J=7.7$ Hz), 6.97 (2H, dd, $J=7.3$, 7.9 Hz), 7.34 (2H, d, $J=7.9$ Hz), 7.77 ppm (2H, s); ^{13}C NMR (125 MHz, CD_3OD): $\delta=$ 173.9, 137.8, 130.1, 128.9, 127.0, 123.1, 122.5, 120.7, 112.4, 107.6, 49.1, 40.3, 36.7 ppm; IR: $\tilde{\nu}=3385$, 2924, 2356, 1755, 1693, 1614, 1530, 1417 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{26}H_{29}N_6O_2$ $[M+H]^+$: 457.2347; found 457.2339.

Compound 4c: ^{1}H NMR (400 MHz, CD_3OD): $\delta=3.21$ (2H, brs), 3.99 (2H, t, $J=5.6$ Hz), 6.60 (2H, dd, $J=7.3$, 7.9 Hz), 6.60 (2H, d, $J=7.9$ Hz), 6.97 (2H, dd, $J=7.3$, 7.9 Hz), 7.33 (2H, d, $J=7.9$ Hz), 7.78 ppm (2H, s); ^{13}C NMR (125 MHz, CD_3OD): $\delta=174.3$, 137.8, 130.2, 128.6, 127.0, 123.1, 122.5, 120.7, 112.5, 107.5, 38.4, 36.6 ppm; IR: $\tilde{\nu}=3389$, 3204, 2333, 1752, 1696, 1615, 1529, 1432, 1401 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{22}H_{19}N_4O_2$ $[M+H]^+$: 371.1503; found 371.1504.

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[1] A recent review: M. A. Fuertes, V. Cepeda, C. Alonso, J. M. Perez, *Chem. Rev.* **2006**, *106*, 2045–2064.
[2] G. Wang, K. M. Vasquez, *Front. Biosci.* **2007**, *12*, 4424–4438.
[3] A. Rich, S. Zhang, *Nat. Rev. Genet.* **2003**, *4*, 566–572.
[4] C. Mao, W. Sun, Z. Shen, N. C. Seeman, *Nature* **1999**, *397*, 144–146.
[5] R. Tashiro, H. Sugiyama, *J. Am. Chem. Soc.* **2005**, *127*, 2094–2097.
[6] A. D'Urso, A. Mammana, M. Balaz, A. E. Holmes, N. Berova, R. Lauceri, R. Purrello, *J. Am. Chem. Soc.* **2009**, *131*, 2046–2047.
[7] Y. Zhang, H. Yu, J. Ren, X. Qu, *Biophys. J.* **2006**, *90*, 3203–3207.
[8] X. G. Qu, J. O. Trent, I. Fokt, W. Priebe, J. B. Chaires, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 12032–12037.
[9] Y. Xu, Y. X. Zhang, H. Sugiyama, T. Umano, H. Osuga, K. Tanaka, *J. Am. Chem. Soc.* **2004**, *126*, 6566–6567.

[10] G. Manzini, L. E. Xodo, F. Quadrifolglio, J. H. van Boom, G. A. van der Marel, *J. Biomol. Struct. Dyn.* **1987**, *5*, 651–662.
[11] F.-M. Chen, *Nucleic Acids Res.* **1988**, *16*, 2269–2281.
[12] D. Bancroft, L. D. Williams, A. Rich, M. Egli, *Biochemistry* **1994**, *33*, 1073–1086.
[13] H. Ohishi, M. Odoko, K. Grzeskowiak, Y. Hiyama, K. Tsukamoto, N. Maezaki, T. Ishida, T. Tanaka, N. Okabe, K. Fukuyama, D. Y. Zhou, K. Nakatani, *Biochem. Biophys. Res. Commun.* **2008**, *366*, 275–280.
[14] A. Rodger, S. Taylor, G. Adlam, I. S. Blagbrough, I. S. Haworth, *Biopolymers* **1994**, *34*, 1583–1593.
[15] A. J. Geall, R. J. Taylor, M. E. Earll, M. A. W. Eaton, I. S. Blagbrough, *Chem. Commun.* **1998**, 1403–1404.
[16] N. Shimada, A. Kano, A. Maruyama, *Adv. Funct. Mater.* **2009**, *19*, 3590–3595.
[17] H. Sugiyama, K. Kawai, A. Matsunaga, K. Fujimoto, I. Saito, H. Robinson, A. H. Wang, *Nucleic Acids Res.* **1996**, *24*, 1272–1278.
[18] S. Ikuta, R. Chattopadhyaya, H. Ito, R. E. Dickerson, D. R. Kearns, *Biochemistry* **1986**, *25*, 4840–4849.
[19] M. M. Patel, T. J. Anchordoquya, *Biophys. Chem.* **2006**, *122*, 5–15.
[20] J. M. Ferreira, R. D. Sheardy, *Biophys. J.* **2006**, *91*, 3383–3389.
[21] P. S. Ho, G. J. Quigley, R. Tilton, A. Rich, *J. Phys. Chem.* **1988**, *92*, 939–945.
[22] T. F. Kagawa, D. Stoddard, G. W. Zhou, P. S. Ho, *Biochemistry* **1989**, *28*, 6642–6651.
[23] T. F. Kagawa, M. L. Howell, K. Tseng, P. S. Ho, *Nucleic Acids Res.* **1993**, *21*, 5978–5986.
[24] R. S. Preisler, H. H. Chen, M. F. Colombo, Y. Choe, B. J. Short, Jr., D. C. Rau, *Biochemistry* **1995**, *34*, 14400–14407.
[25] S. Nakano, L. Wu, H. Oka, H. T. Karimata, T. Kirihata, Y. Sato, S. Fujii, H. Sakai, M. Kuwahara, H. Sawai, N. Sugimoto, *Mol. BioSyst.* **2008**, *4*, 579–588.
[26] P. L. Gilbert, D. E. Graves, M. Britt, J. B. Chaires, *Biochemistry* **1991**, *30*, 10931–10937.
[27] P. A. Mirau, D. R. Kearns, *Nucleic Acids Res.* **1983**, *11*, 1931–1941.
[28] M. Britt, F. Zunino, J. B. Chaires, *Mol. Pharmacol.* **1986**, *29*, 74–80.
[29] C.-W. Chen, R. H. Knop, J. S. Cohen, *Biochemistry* **1983**, *22*, 5468–5471.

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