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Syntheses of nucleic acid mimics designed for metal-induced strand formation on DNA

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Abstract—This paper describes design and syntheses of a new class of chelator-type nucleic acid mimics. Four nucleic acid mimics **7**, **11**, **15**, **17** possess a nucleobase and two ethylenediamine moieties for intermolecular metal coordination. We expected that chelator-type nucleic acid mimics formed a strand in the presence of metal ion and then interacted with a single-stranded DNA. Efficient synthetic routes leading to the four nucleic acid analogues have been achieved via the key intermediate compound **5** which was derived from 3,5-dimethylphenol in five steps. Compound **7** was treated with zinc nitrate and Zn complex **8** was isolated as crystals. ^{1}H NMR study of Zn complex **8** in D_2O showed the single-strand formation. Job plots showed that mixture of $(dT)_{70}$ with Zn complex **8** at 5°C reached a minimum absorbance at a mole ratio of 2:1, and the hypochromic effect showed the $\pi-\pi$ stacking interaction between the nucleobases. Thermal denaturation study at 2:1 mole ratio of complexes between $(dT)_{70}$ and Zn complex **8** showed melting point at 27°C. © 2002 Elsevier Science Ltd. All rights reserved.

1. Introduction

Recently, functionalization of DNA at the molecular level has gained more and more attention from the viewpoint of development of highly controlled materials.[1] Originally, DNA is a bio-informational molecule which codes protein sequences by the order of four nucleotides. The double helical structure of DNA contains complementary hydrogen bonding and π-stacked base pairs. The backbone of each strand is constructed by phosphodiester linkage. Reconstruction of each structural component of DNA is expected to generate a novel type of engineered DNAs with unique structure and high functions.[2]

We envisioned that single-stranded oligonucleotides could function as molecules which potentially record information

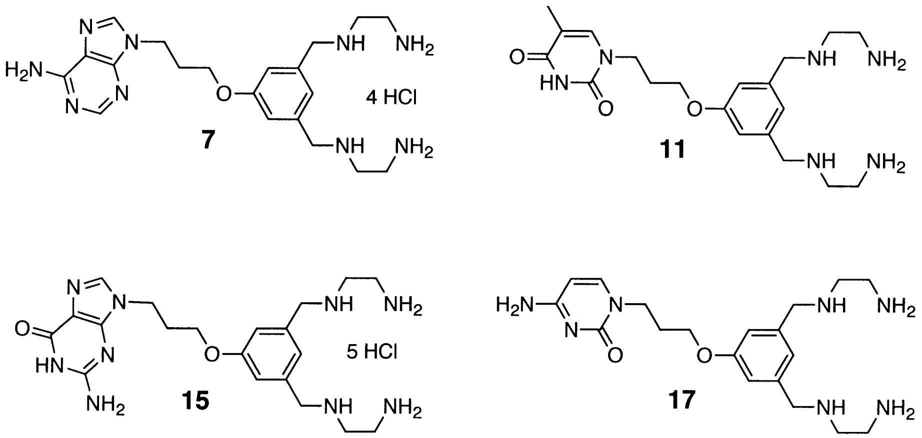

Chart 1.

Keywords: DNA; chelator-type nucleic acid mimics; intermolecular metal coordination.

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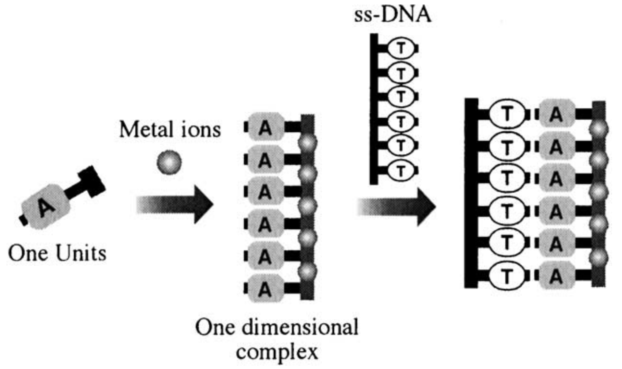
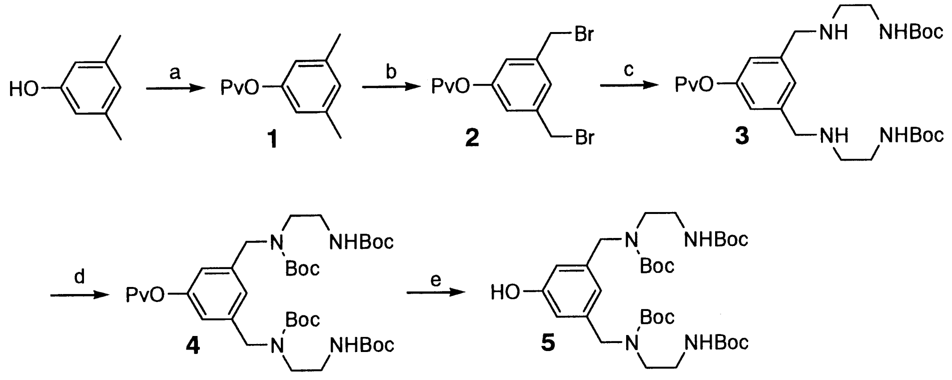


Figure 1. Schematic representation of self-assembly of adenines on a template single-stranded DNA via metal coordination.

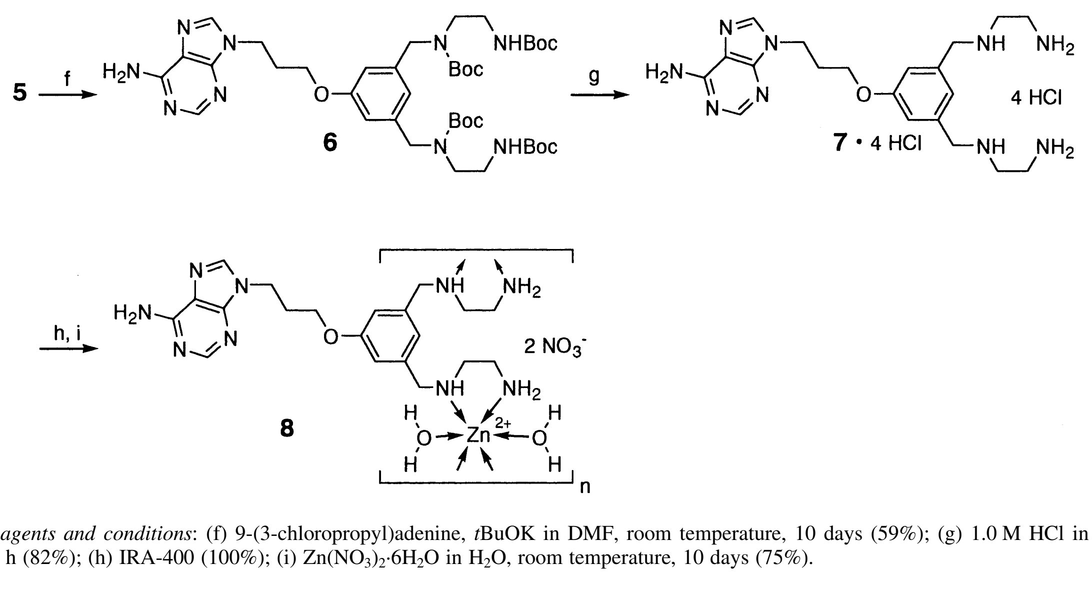
to control the spatial arrangement of metal ions or functional groups. We previously reported the use of a single-stranded DNA template to control the assembly of the complementary adenine mimic **7**,[3,4] which has two metal binding sites and an adenine base moiety. This adenine mimic can be build up into a single-stranded polymer by intermolecular zinc coordination. The single-stranded Zn polymer **8** forms an aggregate with complementary single-stranded $(dT)_{70}$.[3] In this paper, we describe the convenient syntheses of four metal chelator-type nucleic acid mimics **7**, **11**, **15**, **17** (Chart 1), and the interaction of Zn complex **8** with a single-stranded DNA $(dT)_{70}$ (Fig. 1).

2. Results and discussion

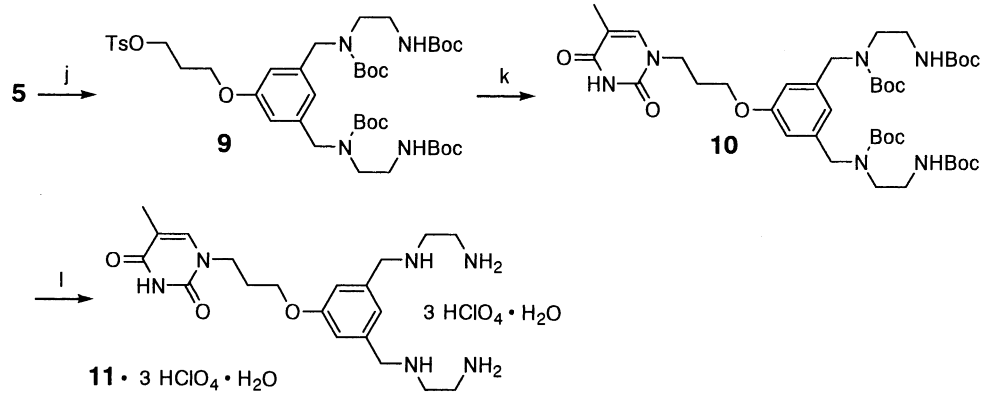
The syntheses of the nucleic acid mimics comprised of a nucleobase and two chelating sites are given in Schemes 1–5. First, the common intermediate compound **5** was synthesized from 3,5-dimethylphenol (Scheme 1). The phenolic hydroxyl group was protected by pivaloyl chloride in CH_2Cl_2 to obtain pivaloyl ester **1**. Bromination of the resulting pivaloyl ester **1** was then carried out by heating with *N*-bromosuccinimide (NBS) in CCl_4 at reflux to afford dibromide **2**.[5] The compound **3** was synthesized by the coupling reaction between compound **2** and *N*-Boc-ethylenediamine.[6] The two amino groups of compound **3** were protected by Boc groups using di-*t*-butyl dicarbonate to afford **4**. Removal of the pivaloyl group of **4** was performed in 1 M NaOH aqueous solution and THF (1:1) to obtain compound **5** in 98% yield, which is the common intermediate for four nucleobase derivatives.



Scheme 1. *Reagents and conditions*: (a) pivaloyl chloride, DMAP, $(iPr)_2NEt$ in CH_2Cl_2, room temperature, 1 h (98%); (b) NBS, benzoylperoxide in CCl_4, reflux, 22 h (58%); (c) $NH_2CH_2CH_2NHBoc$, $(iPr)_2NEt$ in CH_2Cl_2, 50°C, 2 h (45%); (d) di-*t*-butyl dicarbonate, $(iPr)_2NEt$ in CH_2Cl_2, 35°C, 3 h (78%); (e) 1 M NaOH in H_2O, THF, 60°C, 6 h (98%).



Scheme 2. *Reagents and conditions*: (f) 9-(3-chloropropyl)adenine, *t*BuOK in DMF, room temperature, 10 days (59%); (g) 1.0 M HCl in AcOH, room temperature, 1 h (82%); (h) IRA-400 (100%); (i) $Zn(NO_3)_2{\cdot}6H_2O$ in H_2O, room temperature, 10 days (75%).



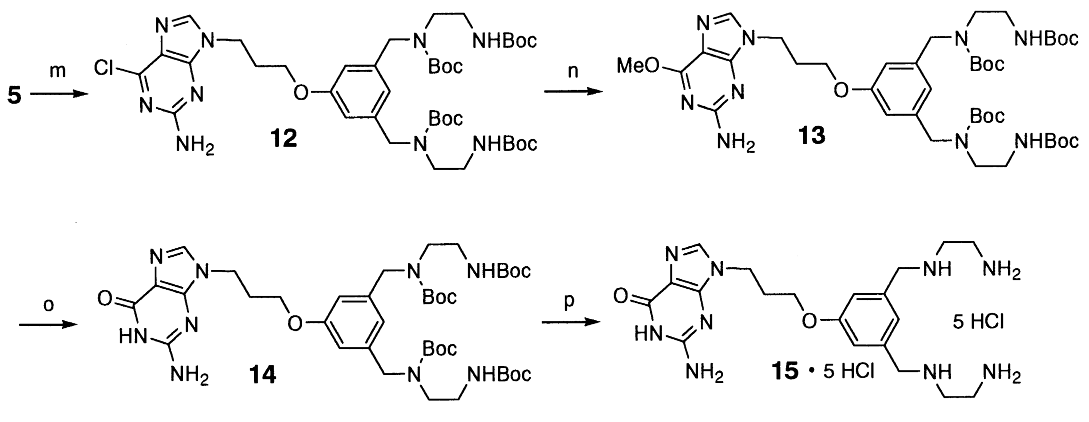
Scheme 3. *Reagents and conditions*: (j) 1,3-bis(p-toluenesulfonyloxy)propane, tBuOK in DMF, room temperature, 1.5 h (76%); (k) 18-crown-6, tBuOK in MeOH, 50°C, 23 h (61%); (l) 1.0 M HCl in AcOH, 0°C, 15 min (82%).

The coupling reaction between **5** and 9-(3-chloropropyl)-adenine was carried out in the presence of potassium t-butoxide (tBuOK) in DMF (Scheme 2)[7] to afford **6** in 71% yield. The tetrahydrochloride salts of compound **7** were obtained by the treatment with 1 M HCl in acetic acid. Ion exchange column chromatography (IRA-400) was carried out to obtain acid-free form **7**.

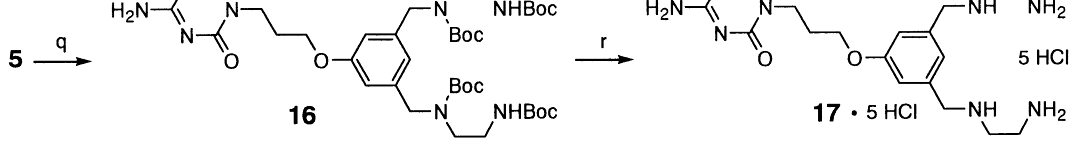
The synthetic route for the thymine derivative **11** was different from that for the adenine form **7**, as shown in Scheme 3. Alkylation of **5** with 1,3-bis(p-toluenesulfonyloxy)propane afforded **9**. The coupling reaction of **9** with thymine was performed in MeOH in the presence of tBuOK and 18-crown-6 (Scheme 3). Deprotection of **10** leading to **11** was then carried out in a manner similar to that for compound **7**.

Since selective alkylation of guanine base at the N9 position was found to be difficult, 2-amino-6-chloropurine was used as the starting material.[8] Alkylation of 2-amino-6-chloropurine with 1-bromo-3-chloropropane afforded 9H-2-amino-6-chloro-9-(3-chloropropyl)purine and 9H-2-amino-6-chloro-7-(3-chloropropyl)purine (N9 alkylated purine–N7 alkylated purine=26:1). Alkylation at the N9-position was evidenced by X-ray crystallography.[9] The coupling between **5** and 9H-2-amino-6-chloro-9-(3-chloropropyl)-purine was carried out in the presence of tBuOK in DMF (Scheme 4). The chloro group of **12** was replaced by the methoxy group (**13**) using sodium methoxide in MeOH, and subsequent treatment with 4 M NaOH in MeOH afforded the guanine form (**14**). The Boc groups were readily removed in 1 M HCl in acetic acid, and then recrystallized from H_2O/EtOH to afford **15**.

The synthesis of the cytosine monomer **17** was performed in a manner similar to that of the adenine form. The coupling between **5** and 3-chloropropylcytosine was carried out in the presence of tBuOK to afford **16** in 30% yield. Deprotection with 1 M HCl in acetic acid afforded compound **17** in 78% yield.



Scheme 4. *Reagents and conditions*: (m) 9H-2-amino-6-chloro-9-(3-chloropropyl)purine, tBuOK in DMF, 60°C, 3 days (71%); (n) MeONa in MeOH, room temperature, 10 min (87%); (o) 4 M NaOH aq. in MeOH, 70°C, 1 week (55%); (p) 1.0 M HCl in AcOH, room temperature, 30 min (37%).



Scheme 5. *Reagents and conditions*: (q) 3-chloropropylcytosine, tBuOK in DMF, 40°C, 10.5 h (30%); (r) 1.0 M HCl in AcOH, 0°C, 15 min (78%).

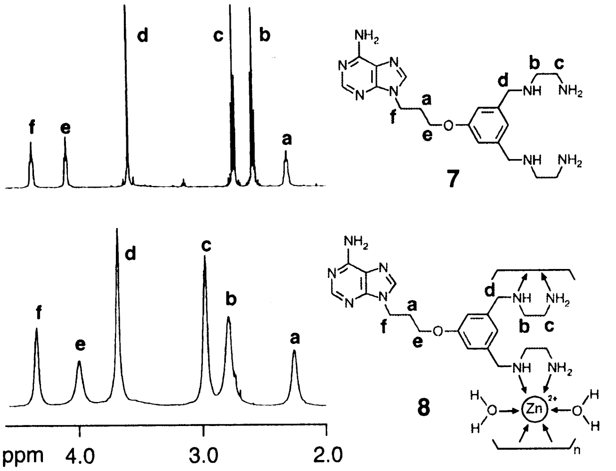


Figure 2. ^{1}H NMR spectra of: (a) compound **7** and (b) Zn complex **8** in D_2O. All signals were assigned by NOESY experiment.

Reaction of **7** with $Zn(NO_3)_2$ in water afforded crystals of $[\mathbf{7}\text{-}Zn^{II}(OH_2)_2](NO_3)_2(H_2O)_{10}$, **8**, by slow evaporation of water. The polymeric structure has been previously determined by single-crystal X-ray diffraction.[3]

The solution behavior of Zn complex **8** was examined by ^{1}H NMR spectroscopy (Fig. 2). The proton resonances of ethylenediamine sites, **b** and **c** shifted to downfield and significantly broadened compared with those of the ligand itself. This observation shows the decrease in electron density around ethylenediamine moieties upon complexation. The broadening of all signals implies the formation of the oligomeric or polymeric structures by intermolecular metal coordination. This result is in good agreement with the polymeric structure clarified by X-ray analysis.[3]

In this study, to determine the ratio of Zn complex **8** to $(dT)_{70}$ in their association form, we carried out Job plotting (Fig. 3). UV absorption change at 260 nm was monitored at 5°C for mixture of Zn complex **8** with $(dT)_{70}$ (total base=30 μM/base in different ratio). As a result, the hypochromicity was maximum at a mole fraction of 0.67 $(dT)_{70}$ to 0.33 Zn complex **8**. This result clearly shows that the presence of triple-stranded complexes are formed by $(dT)_{70}$ and Zn complex **8** in a 2:1 ratio. It is well-known that the formation of triple-stranded complexes $(dA)–(dT)_2$ lead to a hypochromic shift at 280 nm.[10] Hypochromic effects were observed at 280 nm with respect to Zn complex **8** and $(dT)_{70}$. This observation should be consistent with the proposal that complex of Zn complex **8** and $(dT)_{70}$ were three-stranded.

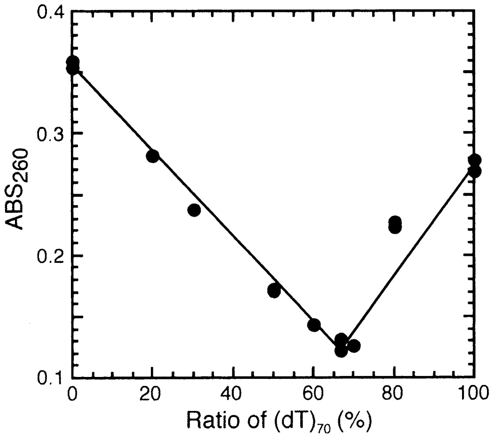


Figure 3. Job plotting for Zn complex **8** and $(dT)_{70}$. 1.0 mM Mops, pH 7.0, 260 nm at 5°C, [Total base]=30 μM/base.

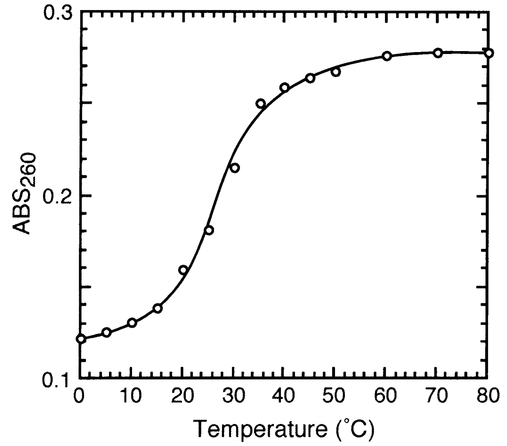


Figure 4. UV melting curves for 2:1 molar ratio of Zn complex **8** and $(dT)_{70}$. $[(dT)_{70}]$=20 μM/base and [Zn complex **8**]=10 μM/base, Mops buffer (1.0 mM, pH 7.0).

The melting curve of a mixture of $(dT)_{70}$ and Zn complex **8** in a 2:1 ratio shows a biphasic transition at 27°C (Fig. 4).[11] It is possible that these results show the formation of the three-stranded complex by hydrogen bonded interactions between Zn complex **8** and $(dT)_{70}$.[12,13]

3. Conclusion

In this study, we have prepared the four chelator-type nucleic acid mimics, and examined the interaction between Zn complex **8** and a natural single-stranded DNA $(dT)_{70}$ by the spectroscopic method. UV melting experiment clearly showed the reversible association and dissociation processes between Zn complex **8** and $(dT)_{70}$. In addition, Job plotting for the extent of hypochromic effects further indicated the possibility of the triple-strand formation consisted of two natural strands and an oligomeric or polymeric Zn complex in aqueous solution with low ionic strength.

4. Experimental

4.1. General

All solvents and reagents were of reagent-grade quality, and used without further purification. TLC analysis was carried out on silica gel 60 F_{254} 1.05554 (Merck). Column chromatography was performed using Wakogel C-300 (silica gel, Wako) or Silica gel 60 (Merck).

^{1}H and ^{13}C NMR spectra were recorded on either a JEOL Lambda 500 or a Bruker DRX-500 spectrometer (500 MHz for ^{1}H; 125.65 MHz for ^{13}C). The spectra were referenced to

TMS in chloroform-*d* or DMSO-d_6, and to TSP in D_2O. Chemical shifts (δ) are reported in ppm; multiplicity is indicated by: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). Coupling constants, *J*, are reported in Hz. FABMS, EIMS, ESIMS were recorded on a Shimadzu KRATOS CONCEPT IS, a Shimadzu QP 1000 EX, and a PE SCIEX API-300 spectrometer, respectively. Melting points were obtained on a Yanaco MP-500D. UV spectra were measured on a Hitachi U-3500 spectrometer in a 1 cm quartz cell.

4.1.1. 3,5-Dimethylphenyl pivaloate (1). To a solution of 3,5-dimethyphenol (25.0 g, 0.21 mol) and 4-dimethylaminopyridine (5.0 g, 0.041 mol), triethylamine (22.3 g, 0.22 mol) in CH_2Cl_2 (40 mL) was added a cooled (ice-water bath) solution of pivaloyl chloride (26.5 g, 0.22 mol) in CH_2Cl_2 (60 mL) dropwise over 1 h with a nitrogen inlet. The reaction mixture was stirred for 1 h at room temperature, and then poured into 600 mL CH_2Cl_2, washed with water three times, and dried over anhydrous $MgSO_4$. After filtration, the solvent was evaporated. Purification of the residue by column chromatography on silica gel with *n*-hexane–ethyl ether (1:1) gave **1** as a colorless syrup (42.5 g, 98%). ^1H NMR ($CDCl_3$): δ 1.34 (9H, s), 2.30 (6H, s), 6.67 (2H, s), 6.84 (1H, s); ^{13}C NMR ($CDCl_3$): δ 177.3, 151.0, 139.2, 127.3, 119.1, 39.0, 27.2, 21.2; EIMS *m/e* 206 (M^+); HRMS calcd for $C_{13}H_{18}O_2$ 206.1307, found 206.1315.

4.1.2. 3,5-Bis(bromomethyl)phenyl pivaloate (2). NBS (78.3 g, 0.44 mol) and benzoyl peroxide (0.10 g) were added in 10 equal portions separately during 6 h to a refluxing solution of 3,5-dimethylphenyl pivaloate (42.2 g, 0.20 mol) in CCl_4 (412 mL). The reaction mixture was further heated at reflux for 16 h, the resulting precipitate was removed by filtration. The residual oil obtained after evaporation was chromatographed on silica gel with *n*-hexane–toluene (3.5:1) and then recrystallized from *n*-hexane to afford **2** as pale yellow needles (42.2 g, 58%). Mp 44.0–45.0°C; ^1H NMR ($CDCl_3$): δ 1.36 (9H, s), 4.45 (4H, s), 7.06 (2H, s), 7.27 (1H, s); ^{13}C NMR ($CDCl_3$): δ 176.8, 151.4, 139.7, 126.7, 122.2, 39.2, 32.0, 27.1; EIMS *m/e* 364 (M^+); HRMS calcd for $C_{13}H_{16}Br_2O_2$ 363.9498, found 363.9511.

4.1.3. Compound 3. To a solution of *N*-Boc-ethylenediamine[6] (17.6 g, 110 mmol) and diisopropylethylamine (1.42 g, 11.0 mmol) dissolved in CH_2Cl_2 (5 mL) was added dropwise a solution of **2** (2.0 g, 5.49 mmol) in CH_2Cl_2 (10 mL) over 1.5 h at room temperature. After 0.5 h, the reaction mixture was poured into CH_2Cl_2 (150 mL), washed with water twice, dried over anhydrous $MgSO_4$, and evaporated. The crude product was chromatographed on silica gel with $CHCl_3$–MeOH (20:1) to obtain **3** as a pale yellow foam (1.79 g, 45%). ^1H NMR ($CDCl_3$): δ 1.35 (9H, s), 1.48 (18H, s), 1.57 (2H, br), 2.75 (4H, br), 3.23 (4H, br), 3.71 (4H, s), 4.96 (2H, br), 6.92 (2H, s), 7.13 (1H, s); ^{13}C NMR ($CDCl_3$): δ 177.2, 156.2, 151.4, 141.9, 129.0, 128.2, 125.3, 125.0, 119.8, 79.2, 53.1, 48.7, 40.2, 39.1, 28.4, 27.2; FABMS *m/e* 523 $(M+H)^+$; HRMS calcd for $C_{27}H_{47}N_4O_6$ 523.3496, found 523.3482.

4.1.4. Compound 4. To a solution of **3** (2.88 g, 5.51 mmol) and diisopropylethylamine (1.56 g, 12.1 mmol) in CH_2Cl_2 (40 mL) was added dropwise a solution of di-*t*-butyl dicarbonate (2.65 g, 12.1 mmol) over 15 min. The reaction mixture was stirred for 3 h at room temperature and then washed with water three times, dried over anhydrous $MgSO_4$, and evaporated. The crude product was chromatographed on silica gel with *n*-hexane–AcOEt (2:1) to obtain **4** as a pale yellow foam (3.11 g, 78%). ^1H NMR ($CDCl_3$): δ 1.35 (9H, s), 1.43 (36H, s), 3.26 (8H, br), 4.41 (4H, br), 4.83 (1H, br), 5.02 (1H, br), 6.82 (2H, br), 6.94 (1H, br); ^{13}C NMR ($CDCl_3$): δ 177.0, 156.1, 155.9, 151.6, 140.3, 123.4, 119.9, 119.2, 80.4, 79.1, 50.6, 50.0, 46.0, 39.1, 39.1, 28.4, 27.1; FABMS *m/e* 724 $(M+H)^+$; HRMS calcd for $C_{37}H_{63}N_4O_{10}$ 723.4544, found 723.4540.

4.1.5. Compound 5. To a solution of **4** (1.94 g, 2.69 mmol) dissolved in THF (20 mL) was added 1.0 M NaOH aqueous solution (20 mL). The reaction mixture was heated at 60°C for 6 h. Then the solution was neutralized with Dowex 50W-X8 (Pyridinium form). The resin was removed by filtration and the filtrate was concentrated on a rotary evaporator. The crude material purified by silica gel chromatography using *n*-hexane–AcOEt (2:1) afforded **5** as a pale yellow foam (1.72 g, 100%). ^1H NMR ($CDCl_3$): δ 1.43 (36H, s), 3.32 (8H, br), 4.44 (4H, br), 5.01 (1H, br), 5.16 (1H, br), 6.55 (1H, s), 6.60 (2H, s); ^{13}C NMR ($CDCl_3$): δ 157.4, 156.6, 156.3, 140.1, 139.8, 113.5, 113.2, 80.4, 79.3, 50.8, 50.1, 45.8, 39.2, 28.4, 27.2; FABMS *m/e* 637 $(M-H)^-$; HRMS calcd for $C_{32}H_{53}N_4O_9$ 637.3813, found 637.3800.

4.1.6. Compound 6. To a solution of **5** (1.63 g, 2.55 mmol) and *t*BuOK (0.35 g, 3.07 mmol) dissolved in DMF (50 mL) was added 9-(3-chloropropyl)adenine (0.48 g, 2.55 mmol) in one portion. The reaction mixture was heated at 50°C for 10 days and then the solvent was concentrated on a rotary evaporator. The residue was dissolved into 80 mL CH_2Cl_2 and then washed with water twice, and dried over anhydrous $MgSO_4$, the solvent was removed on a rotary evaporator. The crude material was purified by silica-gel column chromatography with $CHCl_3$–MeOH (25:1) to afford **6** as a pale yellow foam (1.08 g, 59%). ^1H NMR ($CDCl_3$): δ 1.43 (36H, s), 2.36 (2H, q, J=6.2 Hz), 3.22 (8H, br), 3.93 (2H, t, J=5.6 Hz), 4.38 (4H, s), 4.42 (2H, t, J=6.7 Hz), 4.94 (1H, br), 5.61 (1H, br), 6.61 (2H, s), 6.69 (1H, s), 7.73 (1H, s), 8.33 (1H, s); FABMS *m/e* 815 $(M+H)^+$; HRMS calcd for $C_{40}H_{64}N_9O_9$ 814.4827, found 814.4867.

4.1.7. Compound 7·4HCl. Compound **6** (1.01 g, 1.24 mmol) was placed in a flask, to which 1.0 M HCl in acetic acid (40 mL) was added. The solution was stirred in an ice-water bath for 1 h. The solvent was removed in vacuo and the resulting colorless solid was recrystallized from H_2O/*i*PrOH to afford **7**·4HCl as colorless needles (0.569 g, 82%). Mp 249.0–250.5°C; ^1H NMR (D_2O): δ 2.26 (2H, q, J=6.1 Hz), 3.27 (4H, t, J=5.7 Hz), 3.31 (4H, t, J=5.6 Hz), 4.01 (2H, t, J=5.6 Hz), 4.15 (4H, s), 4.40 (2H, t, J=6.6 Hz), 6.86 (2H, s), 7.01 (1H, s), 8.17 (1H, s), 8.20 (2H, s); ESIMS *m/e* 414 $(M)^+$, *m*/2*e* 207 $(M)^+$.

4.1.8. Compound 7. The HCl salts of **7** (150 mg, 0.268 mmol) were passed through an ion exchange column

(IRA-400, Organo Co. Japan) to obtain acid-free **7**, which was recrystallized from MeCN to afford **7** as colorless prisms (110 mg, 99%). Mp 109.5–110.5°C; ^{1}H NMR (D_2O): δ 2.30 (2H, q, *J*=5.4 Hz), 2.56 (4H, t, *J*=6.6 Hz), 2.73 (2H, t, *J*=6.6 Hz), 3.58 (4H, s), 4.09 (2H, t, *J*=4.8 Hz), 4.36 (2H, t, *J*=5.2 Hz), 6.29 (2H, s), 6.75 (1H, s), 7.86 (1H, s), 8.08 (1H, s); ^{13}C NMR (D_2O): δ 158.9, 156.5, 153.1, 150.3, 143.9, 141.2, 122.3, 119.7, 114.4, 67.4, 53.1, 47.4, 43.4, 40.0, 29.4; ESIMS *m*/*e* 414 $(M)^+$, *m*/2*e* 207 $(M)^+$; Anal. calcd for $C_{20}H_{31}N_9O$: C, 58.09; H, 7.56; N, 30.48. Found: C, 57.72; H, 7.34; N, 30.07.

4.1.9. Zn complex 8. To a solution of **7** (22.8 mg, 55.1 μmol) dissolved in distilled water (100 μL) was added 0.5 M $Zn(NO_3)_2$ aqueous solution (110 μL). The reaction mixture was filtered and the filtrate was slowly evaporated to obtain the Zn complex **8** as colorless prisms (26.4 mg, 75%). ^{1}H NMR (D_2O): δ 1.91 (2H, br), 2.77 (4H, br), 2.96 (2H, br), 3.67 (4H, s), 3.99 (2H, br), 4.34 (2H, br), 6.37 (2H, s), 6.79 (1H, s), 7.88 (1H, s), 8.06 (1H, s); Anal. calcd for $C_{20}H_{39}N_{11}O_9Zn$: C, 37.60; H, 5.52; N, 24.11. Found: C, 37.48; H, 5.65; N, 23.87 (for the sample completely dried).

4.1.10. Compound 9. To a solution of **5** (2.03 g, 3.17 mmol) and *t*BuOK (0.426 g, 3.80 mmol) dissolved in DMF (6 mL) was added 1,3-di(*p*-toluenesulfonyloxy)propane (3.66 g, 5.73 mmol) in one portion and the reaction mixture was stirred for 25 min at room temperature. After 1.5 h the solvent was removed in vacuo. The residue was dissolved in 150 mL CH_2Cl_2 and then washed with water twice, and dried over anhydrous $MgSO_4$, the solvent was removed on a rotary evaporator. The crude material was purified by silica-gel column chromatography with *n*-hexane–AcOEt (3:1) to afford **9** as a pale yellow foam (2.06 g, 76%). ^{1}H NMR ($CDCl_3$): δ 1.43 (36H, s), 2.10 (2H, br), 2.41 (3H, s), 3.24 (8H, br), 3.94 (2H, br), 4.23 (2H, t, *J*=6.1 Hz), 4.37 (4H, br), 4.74 (1H, br), 5.00 (1H, br), 6.58 (2H, s), 6.65 (1H, br), 7.30 (1H, d, *J*=7.3 Hz), 7.77 (2H, d, *J*=7.7 Hz); FABMS *m*/*e* 851 $(M)^+$.

4.1.11. Compound 10. To a solution of thymine (0.355 g, 2.66 mmol), 18-crown-6 (0.767 g, 2.90 mmol), and *t*BuOK (0.325 g, 0.290 mmol) in MeOH (5 mL) was added dropwise a solution of **9** (2.06 g, 2.42 mmol) in MeOH (10 mL) over 5 min under argon and at room temperature. The reaction mixture was stirred for 23 h at 50°C. After evaporation of the solvent, the residue was poured into 150 mL CH_2Cl_2, and the solution was washed with water twice, dried over anhydrous $MgSO_4$, and concentrated. The crude material was purified by silica-gel column chromatography with *n*-hexane–AcOEt (1:3) to afford **10** as a pale yellow foam (1.19 g, 61%). ^{1}H NMR ($CDCl_3$): δ 1.43 (36H, s), 1.87 (3H, s), 2.15 (2H, br), 3.25 (8H, br), 3.91 (2H, t, *J*=6.7 Hz), 3.99 (2H, t, *J*=5.5 Hz), 4.39 (4H, br), 4.82 (1H, br), 5.04 (1H, br), 6.63 (1H, s), 6.68 (2H, s), 7.02 (1H, s), 8.67 (1H, br); ^{13}C NMR ($CDCl_3$): δ 164.0, 159.0, 156.2, 155.9, 150.8, 140.9, 140.5, 140.2, 112.7, 112.2, 110.6, 80.3, 79.4, 63.7, 50.8, 50.2, 46.1, 39.1, 29.7, 28.4, 12.3, FABMS *m*/*e* 806 $(M+H)^+$; HRMS calcd for $C_{40}H_{65}N_6O_{11}$ 805.4711, found 805.4748.

4.1.12. Compound 11·3HClO$_4$·H$_2$O. Compound **10** (0.756 g, 0.939 mmol) was placed in a flask, to which 1.0 M HCl in acetic acid (20 mL) was added. The solution was stirred in an ice-water bath for 15 min. The solvent was evaporated in vacuo. The residue was then dissolved in 1.0 M $NaClO_4$ aqueous solution (7.5 mL), and added to *i*PrOH (5 mL). Recrystallization from H_2O/*i*PrOH afforded **11** as colorless needles (0.424 g, 82%). ^{1}H NMR (D_2O): δ 2.11 (2H, q, *J*=5.9 Hz), 3.35 (8H, m), 3.88 (2H, t, *J*=6.3 Hz), 4.11 (2H, t, *J*=5.5 Hz), 4.22 (4H, s), 7.01 (2H, s), 7.08 (1H, s), 7.39 (1H, s); ^{13}C NMR (D_2O): δ 167.0, 158.9, 152.3, 143.5, 132.8, 123.5, 117.1, 110.6, 66.2, 51.1, 46.6, 43.9, 35.4, 26.9, 11.1; ESIMS *m*/*e* 405 $(M)^+$, *m*/2*e* 202 $(M)^{2+}$; Anal. calcd for $C_{20}H_{37}Cl_3N_6O_{16}$: C, 33.18; H, 5.15; N, 11.61. Found: C, 33.63; H, 5.63; N, 11.55.

4.1.13. Compound 12. To a solution of **5** (150 mg, 0.24 mmol) and *t*BuOK (28 mg, 0.25 mmol) dissolved in DMF (2 mL) was added 9*H*-2-amino-6-chloro-9-(3-chloropropyl)purine (61 mg, 0.25 mmol) in one portion. The reaction mixture was heated at 60°C for 3 days and then the solvent was concentrated on a rotary evaporator. The residue was dissolved in 80 mL CH_2Cl_2 and then washed with water twice, dried over anhydrous $MgSO_4$, and the solvent was removed on a rotary evaporator. The crude material was purified by silica-gel column chromatography with $CHCl_3$–MeOH (25:1) to afford **12** as a pale yellow foam (1.48 g, 71%). ^{1}H NMR ($CDCl_3$): δ 1.43 (36H, s), 2.31 (2H, br), 3.26 (8H, br), 3.93 (2H, t, *J*=5.6 Hz), 4.32 (2H, t, *J*=6.5 Hz), 4.40 (4H, br), 4.85 (1H, s), 5.07 (1H, s), 5.31 (2H, s), 6.60 (1H, s), 6.67 (2H, s), 7.75 (1H, s).

4.1.14. Compound 13. To a solution of **12** (0.99 g, 1.16 mmol) dissolved in MeOH (5 mL) was added dropwise a solution of 28% sodium methoxide in MeOH (2.4 mL, 11.8 mmol) over 10 min under argon at room temperature. The reaction mixture was stirred at room temperature for 0.5 h, and then added to 120 mL CH_2Cl_2, washed with brine three times, dried over anhydrous $MgSO_4$ and concentrated. Product **13** was obtained as a colorless syrup (0.584 g, 87%) and used without further purification for next reaction. ^{1}H NMR ($CDCl_3$): δ 1.43 (36H, s), 2.30 (2H, s), 3.25 (8H, br), 3.91 (2H, t, *J*=5.6 Hz), 4.08 (3H, s), 4.29 (2H, t, *J*=6.5 Hz), 4.39 (4H, br), 4.79 (1H, s), 4.88 (2H, s), 5.03 (1H, s), 6.61 (1H, s), 6.67 (2H, s), 7.56 (1H, s).

4.1.15. Compound 14. To a solution of **13** (0.854 g, 1.01 mmol) dissolved in MeOH (8 mL) was added dropwise 4.0 M NaOH aqueous solution (8 mL) over 1 min under argon at room temperature. The reaction mixture was stirred at 70°C for 1 week, and then poured into 120 mL CH_2Cl_2, washed with water three times, and dried over anhydrous $MgSO_4$. After evaporation, the crude material was purified by silica-gel column chromatography with $CHCl_3$–MeOH (25:1) to afford **14** as pale yellow foam (0.461 g, 55%). ^{1}H NMR ($CDCl_3$): δ 1.42 (36H, s), 2.27 (2H, br), 3.23 (8H, br), 3.94 (2H, t, *J*=5.3 Hz), 4.23 (2H, t, *J*=6.2 Hz), 4.39 (4H, br), 4.87 (1H, br), 5.11 (1H, br), 5.82 (2H, br), 6.61 (3H, m), 7.58 (1H, s), 11.8 (1H, br); ^{13}C NMR ($CDCl_3$): δ 159.2, 159.0, 156.2, 153.6, 152.0, 140.3, 138.0, 117.3, 80.3, 79.2, 64.4, 50.9, 50.3, 46.2, 40.6, 39.3, 29.6, 28.5.

4.1.16. Compound 15·5HCl. Compound **14** (0.461 g, 0.56 mmol) was placed in a flask, to which 1.0 M HCl in acetic acid (10 mL) was added. After stirring for 30 min at room temperature, the reaction mixture was poured into diethyl ether (10 mL) and the resulting colorless precipitate was collected. The crude product was recrystallized from H_2O/EtOH to afford **15** as colorless needles (0.126 g, 37%). ^1H NMR (D_2O): δ 2.19 (2H, q, J=5.2 Hz), 3.35 (8H, br), 4.08 (2H, t, J=5.9 Hz), 4.14 (4H, s), 4.18 (2H, t, J=5.8 Hz), 6.67 (2H, s), 6.97 (1H, s), 7.71 (1H, s); ^{13}C NMR (D_2O): δ 158.7, 158.6, 153.5, 151.9, 140.6, 132.8, 123.3, 116.6, 115.8, 66.6, 51.1, 44.0, 42.1, 35.6, 28.0; Anal. calcd for $C_{20}H_{36}Cl_5N_9O_2$: C, 39.26; H, 5.93; N, 20.60. Found: C, 39.04; H, 5.91; N, 20.40.

4.1.17. Compound 16. To a solution of **5** (150 mg, 0.24 mmol) and *t*BuOK (28 mg, 0.25 mmol) dissolved in DMF (2 mL) was added 3-chloropropylcytosine (61 mg, 0.25 mmol) in one portion. The reaction mixture was heated at 60°C for 3 days and then the solvent was concentrated on a rotary evaporator. The residue was dissolved in 80 mL CH_2Cl_2 and then washed with water twice, and dried over anhydrous $MgSO_4$, the solvent was removed on a rotary evaporator. The crude material was purified by silica-gel column chromatography with $CHCl_3$–MeOH (25:1) to afford **16** as a pale yellow foam (55.1 mg, 30%). ^1H NMR ($CDCl_3$): δ 1.42 (36H, br), 2.20 (2H, br), 3.22 (8H, br), 3.94 (4H, br), 4.38 (4H, br), 4.88 (1H, s), 5.13 (1H, s), 5.69 (1H, d, J=6.6 Hz), 6.65 (3H, br), 7.20 (1H, d, J=6.9 Hz); ^{13}C NMR ($CDCl_3$): δ 166.2, 159.1, 156.6, 156.0, 146.2, 140.3, 118.9, 112.4, 93.9, 80.3, 79.2, 64.5, 51.0, 47.7, 46.2, 39.2, 28.6.

4.1.18. Compound 17·5HCl. To a solution of **16** (55.1 mg, 0.068 mmol) was added 1.0 M HCl in acetic acid (1 mL). The reaction mixture was stirred in an ice-water bath for 15 min and then poured into diethyl ether (5 mL). The resulting colorless precipitate was collected and the filtrate was evaporated in vacuo to afford **17** as a white precipitate (23.1 g, 78%). ^1H NMR (D_2O): δ 2.11 (2H, q, J=6.2 Hz), 3.31 (4H, t, J=6.9 Hz), 3.38 (4H, t, J=6.9 Hz), 3.95 (2H, t, J=6.7 Hz), 4.07 (2H, t, J=5.6 Hz), 4.21 (4H, s), 6.03 (1H, d, J=6.7 Hz), 7.05 (2H, s), 7.09 (1H, s), 7.75 (1H, d, J=6.7 Hz).

4.2. UV measurement and Job plotting (mixing curves)

In the temperature-dependence of UV absorbance at 260 nm, a solution containing an equimolar concentration (15 μM/base) of $(dT)_{70}$ and Zn complex **8** in 1.0 mM Mops buffer at pH 7.0 was used for the measurement. The cuvette-holding chamber was flushed with a constant stream of N_2 gas to avoid water condensation on the cuvette exterior. To obtain the Job plotting for UV absorbance at 260 nm, solutions of $(dT)_{70}$ and Zn complex **8** (total base concentrations were 30 μmol/L) were mixed in a variety of concentrations in 1.0 mM Mops buffer at pH 7.0. Each mixture was heated at 80°C for 20 min and then slowly cooled to 0°C over 3 days.

4.3. Single-crystal X-ray analysis

9*H*-2-Amino-6-chloro-9-(3-chloropropyl)purine. A single crystal ($0.50\times0.10\times0.05$ mm^3) grown from *i*PrOH was used for the unit-cell determinations and the data collections by Rigaku RAXIS-IV imaging plate diffractometer with graphite monochromated Mo Kα radiation. For crystal data of 9*H*-2-amino-6-chloro-9-(3-chloropropyl)purine, see Ref. 9.

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References

1. (a) Tanaka, K.; Okahata, Y. *J. Am. Chem. Soc.* **1996**, *118*, 10679–10683. (b) Mirkin, C. A.; Letsinger, R. L.; Mucic, R. C.; Storhoff, J. J. *Nature* **1996**, *382*, 607–609. (c) Adleman, L. M. *Science* **1994**, *266*, 1021–1023. (d) Storhoff, J. J.; Mirkin, C. A. *Chem. Rev.* **1999**, *99*, 1849–1862. (e) Orgel, L. E. *Acc. Chem. Res.* **1995**, *28*, 109–118. (f) Bag, B. G.; von Kiedrowski, G. *Pure Appl. Chem.* **1996**, *68*, 2145–2152. (g) Kool, E. T. *Chem. Rev.* **1997**, *97*, 1473–1487. (h) Nielsen, P. E. *Acc. Chem. Rev.* **1999**, *32*, 624–630.
2. (a) Arya, D. P.; Bruice, T. C. *J. Am. Chem. Soc.* **1998**, *120*, 12419–12427. (b) Dempcy, R. O.; Browne, K. A.; Bruice, T. C. *J. Am. Chem. Soc.* **1995**, *117*, 6140–6141. (c) Dempcy, R. O.; Almarson, Ö.; Bruice, T. C. *Proc. Natl Acad. Sci. USA* **1994**, *91*, 7464–7868. (d) Fathi, R.; Huang, Q.; Syi, J.-L.; Delaney, W.; Cook, A. F. *Bioconjugate Chem.* **1994**, *5*, 47–57. (e) Dempcy, R. O.; Browne, K. A.; Bruice, T. C. *Proc. Natl Acad. Sci. USA* **1995**, *92*, 6097–6101. (f) Browne, K. A.; Dempcy, R. O.; Bruice, T. C. *Proc. Natl Acad. Sci. USA* **1995**, *92*, 7051–7055.
3. Hatano, A.; Tanaka, K.; Shiro, M.; Shionoya, M. *Chem. Lett.* **2000**, 822–823.
4. Hatano, A.; Morishita, H.; Tanaka, K.; Shionoya, M. *Nucl Acids Symp. Ser.* **1998**, *39*, 171–172.
5. (a) Tashiro, M.; Yamato, T. *J. Org. Chem.* **1985**, *50*, 2939–2942. (b) Vinod, T.; Hart, H. *J. Org. Chem.* **1990**, *55*, 881–890.
6. Nuller, D.; Zeltser, I.; Bitan, G.; Gilon, C. *J. Org. Chem.* **1997**, *62*, 411–417.
7. When 9-(3-bromopropyl)adenine was used instead as an alkylating agent, an intramolecular cyclization product was mainly obtained. See: (a) Itahara, T. *Bull. Chem. Soc. Jpn* **1997**, *70*, 2239–2247. Browne, D. T.; Eisinger, J.; Leonard, N. J. *J. Am. Chem. Soc.* **1968**, *90*, 7302–7323.
8. Geen, G. R.; Grinter, T. J.; Kincey, P. M.; Jarvest, R. L. *Tetrahedron* **1990**, *46*, 6903–6914.
9. Crystal data for 9*H*-2-amino-6-chloro-9-(3-chloropropyl)-purine; $C_8H_9Cl_2N_5$, FW=246.10, prismatic, space group *Aba* (#41), a=23.989(2), b=23.607, c=7.2819(4) Å, Z=16, D_{calcd}=1.585 g/cm^3, ((Mo Kα)=6.01 cm^{-1}, $F_{(000)}$=2016.00, R=0.123, R_w=0.133, R_1=0.067.
10. Riley, M.; Maling, B.; Chamberlin, M. *J. Mol. Biol.* **1966**, *20*, 359–389.
11. The melting curve of a 1:1 mixture of Zn complex **8** with $(dT)_{70}$ was similar to that of natural DNA duplex.
12. In this ratio, the formed complex should be neutral, so that we could not exclude the possibility of the formation of 2:1 poly-ion complex of $(dT)_{70}$ with Zn complex **8**. In the thermal denaturation analysis of a mixture of natural DNA $(dA)_{70}$

and $(dT)_{70}$ in a 1:1 ratio in 100 mM NaCl, plots of absorbance at 260 nm vs temperature exhibited a transition point at 59°C. However, the hypochromic effect was not observed in this case without adding NaCl. On the other hand, since Zn complex **8** possesses positive charges, it possibly interacts with negatively charged DNA strands more strongly in solution with lower ionic strength. Indeed, in the case of Zn complex **8** with $(dT)_{70}$ in a 1:1 ratio, a biphasic transition was observed at 40°C when [NaCl]=0, whereas no transition when [NaCl]=100 mM.

13. In the thermal denaturation analysis of mixtures containing Zn complex **8** and $(dA)_{70}$, $(dG)_{70}$, or $(dC)_{70}$ in a 1:1 ratio, precipitation occurred in 1.0 mM Mops (pH 7.0).