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Synthesis and biological evaluation of the Zn (II)–IDB complexes appended with oligopolyamide as potent artificial nuclease

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ABSTRACT

The zinc (II) complexes, which contain oligopolyamide and bis(2-benzimidazolylmethyl)amine (IDB) conjugated by flexible linker, have been successfully synthesized, characterized, and evaluated as DNA cleavage agents. The cleavage activity of these complexes on DNA was studied by electrophoresis. The results showed that the cleavage activity of zinc (II) complexes was enhanced comparing with those without oligopolyamide. Specially, at a high reaction concentration (1.2 mM), Zn (II) complex can cleave the plasmid DNA bearing some selectivity. Further, the spectroscopic data suggested that Zn (II) complexes with oligopolyamide backbone possessed A-T (adenine and thymine) rich sequences preference.

There is an increasing interest in the realization of small and robust artificial DNA hydrolytic agents for their potential applications not only in molecular biology but also in the development of new drugs, recently.[1] Within these artificial nucleases, several examples of synthetic systems, based on metal complexes, which promote the hydrolysis of nucleic acids or of model phosphate esters have been reported in the past few years.[2–13] However, almost all these systems employ metal ions different from those used by the natural catalysts and, in particular, are often based on lanthanide ions[5–7,14] or Cu (II).[8–13] Although transition metal and lanthanide complexes are shown to be most active for degradation of DNA or RNA, they are highly labile, and the precise coordination number and geometry are difficult to determine.[15] Furthermore, among the physiologically relevant metal ions, Zn (II) is probably the best suited metal ion for the development of artificial metallonucleases.[16] However, its reactivity, especially mononuclear Zn (II) complex, is somewhat lower than that of the other commonly employed transition-metal ions,[15] and this is probably why the examples of Zn (II)-based artificial nucleases reported to date are scarce.[17–22]

As mentioned previously, we expect that the catalytic activity of the small molecular mononuclear Zn (II) complexes is enhanced through some modificators. Since the principal rules developed by Dervan et al. are that antiparallel pairing of Py/Im (Py = *N*-methylpyrrole, Im = *N*-methylimidazole) targets a C-G (cytosine and guanine) base pair, Im/Py targets a G-C base pair; and Py/Py is degenerate, recognizing either an A-T or T-A base pair,[23–25] this makes polyamide system attractive candidate for developing selective and efficient artificial nuclease due to their ability to bind to the minor groove of duplex DNA. Here, we report synthesis, characterization and biological evaluation of a class of Zn (II) complexes **2** which contain oligopolyamide and bis(2-benzimidazolylmethyl)amine conjugated by flexible linker (Scheme 1). In a detailed set of experiments, we found that the Zn (II) complexes can bind to and cleave duplex DNA efficiently, bearing some selectivity. The minimum of reaction concentration and time decrease remarkably compared with the metal complexes without oligopolyamide backbone.

Initially, we focused on the construction of oligopolyamide backbone. The synthesis of these molecular contained 2–4 pyrrolecarboxamide units should be achieved by the 1-hydroxybenzotriazole (HOBt) and *N*,*N*-dicyclohexylcarbodiimide (DCC) coupling reaction. According to the reference,[26] we synthesized a series of oligopolyamide backbone (compounds **3a–c**). Subsequently, compounds **3a–c** were converted into their activated esters with HOBt and DCC in DMF. And then, IDB was added to the above solution with stirring in room temperature for overnight. Compounds **4a–c** were obtained in 78%, 75%, and 70% yields from their precursors, respectively. These ligands were purified by a silica gel column with $CH_3OH/CHCl_3$ (4:96, 4:96, 5:95, respectively) as eluent. In the last step, the free ligands **4a–c** were allowed to react with Zn

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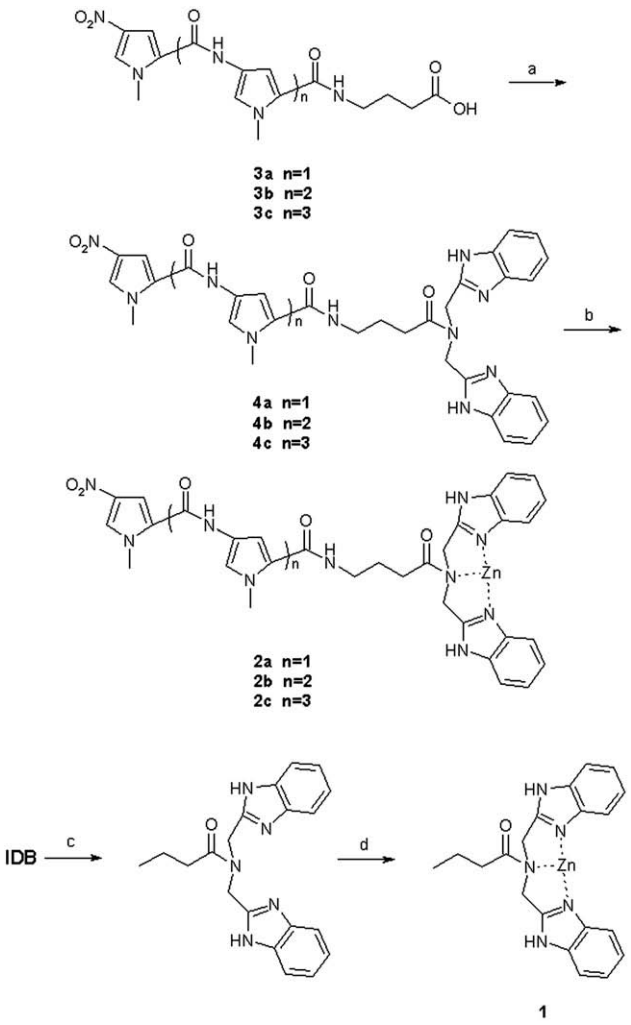

Scheme 1. Reagents conditions and yields: (a) HOBt, DCC, DMF 8 h, rt, and then IDB 8 h (70%, 60% and 62% for **4a**–**c**, respectively). (b) Zn $(ClO_4)_2{\cdot}6H_2O$, CH_3OH, rt, 6 h (78%, 75%, and 72% for **2a**–**c**, respectively). (c) $CH_2CH_2CH_2COCl$, CH_3COCH_3, 0 °C, 10 h (95%); (d) Zn $(ClO_4)_2{\cdot}6H_2O$, CH_3OH, rt, 6 h (76%).

$(ClO_4)_2{\cdot}6H_2O$ in methanol. After stirring in room temperature for 6 h and filtration, the Zn (II) complexes **2a–c** were obtained as yellow solid in 72–79% yields. The compounds **2a–c** and **4a–c** were characterized by IR, ESI-MS, ^{1}H NMR, and HRMS.[27] In order to better understand the important role of oligopolyamide backbone, the control (compound **1**) was synthesized. The route of synthesis was shown in Scheme 1.

The DNA cleavage ability of Zn (II) complexes was initially studied by monitoring the conversion of circular supercoiled DNA (Form I), circular relaxed DNA (Form II) and linear DNA (Form III). The pUC18 plasmid DNA was used as the reaction substrate. We found optimal condition of cleavage reaction through a serious of optimize experiment, including pH value, concentration, time, and temperature. To demonstrate the superiority over the metal complexes without oligopolyamide, contrast experiment was performed under the same conditions which have been optimized.

Figure 1 suggested the effect of different cleavage agents under the optimized conditions. Zn (II) complex **1** at the lower concentration did not result in any detectable cleavage. The plasmid DNA was cleaved by **1** until the concentration up to 1.8 mM, but without any selectivity. However, the efficient cleavage of plasmid DNA in the presence of Zn (II) complex with oligopolyamide **2c** was apparent when the concentration was just at a lower level. The linear DNA (Form III) was observed in agarose gel electrophoresis diagram when 0.6 mM cleavage agent was added to the reaction system, as shown in Figure 1b. The minimum of cleavage concentration lowered apparently. Notably, the disappearance of circular relaxed and linear DNA was accompanied by appearance of the new band when the reaction concentration reached 1.2 mM. The molecular weight of this band was about 1000 bp. It was a convincing argument that the oligopolyamide system played an important role in cleavage activity and selectivity. The metal complex with oligopolyamide and the result of cleavage reaction, have not yet, to the best of our knowledge, been reported in the literature.

Subsequently, our efforts focused on the minimum of reaction time of complexes. The experiment was performed in a similar way. The plasmid DNA was not cleaved by complex **1** in short time (Fig. 2a). The efficient cleavage was observed when the reaction time was reached about 8 h. However, in the event that the complex with oligopolyamide **2c** was added to the reaction at the same

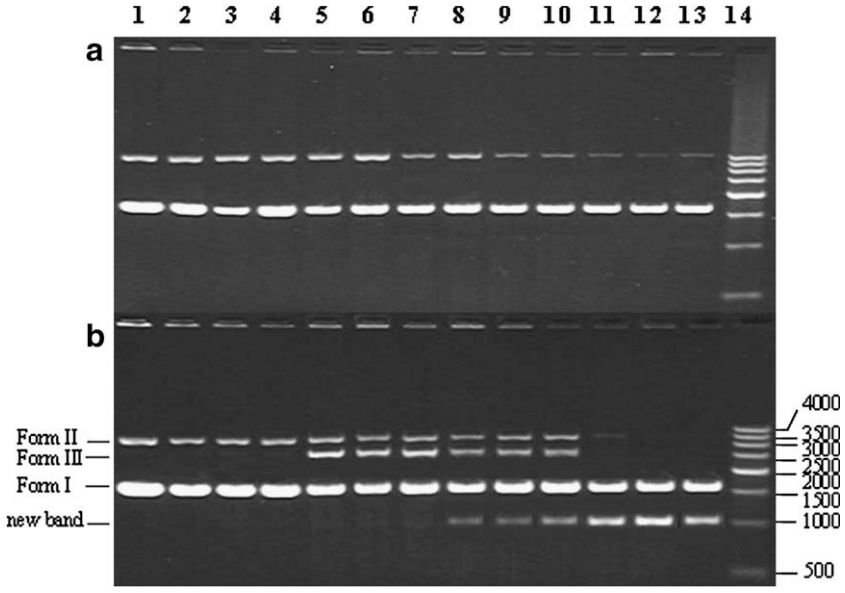

Figure 1. Agarose gel electrophoresis of cleavage reaction of pUC18 DNA (0.08 μg/μl) by different concentrations and complexes (a: **1**, b: **2c**) in a Tris–HCl buffer (40 mM, pH 8.92) for 6 h at 50 °C. Lane 1: DNA control; Lane 2–13: 0.05, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2 mM; Lane 14: 500 bp DNA ladder.

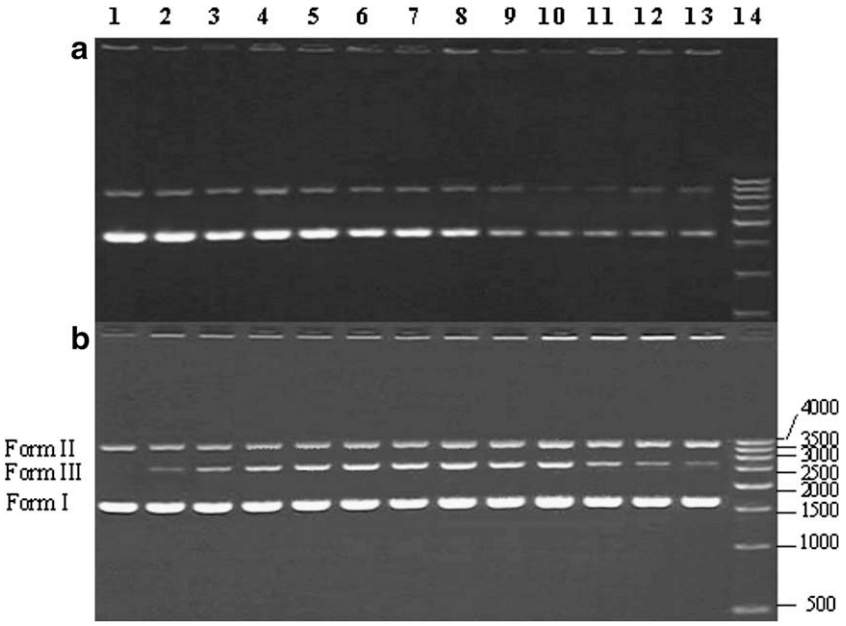

Figure 2. Agarose gel electrophoresis of cleavage reaction of pUC18 DNA (0.08 μg/μl) by different time and complexes (a: **1**, b: **2c**) (0.6 mM) in a Tris–HCl buffer (40 mM, pH 8.92) at 50 °C. Lane 1: DNA control; Lane 2–13: 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 h; Lane 14: 500 bp DNA ladder.

condition, the linear DNA (Form III) was caught when the reaction time was just 30 min (Fig. 2b). The result suggested that the minimum of reaction time lowered sharply in the presence of Zn (II) complex with oligopolyamide.

Further, the influence of the length of the oligopolyamide backbone on the cleavage reaction was studied. As shown in Figure 3a, the amount of linear DNA (Form II) observed in agarose gel electrophoresis diagram increased with increase of pyrrole unit in complexes and compound **2c** is the best catalyst for DNA cleavage. The similar result was observed at high concentration (Fig. 3b). The data suggested that the construction of the complexes, in particular the length of the oligopolyamide backbone appended with IDB group, play an important role in cleavage reaction.

To demonstrate that the cleavage activity was not restricted to plasmid DNA, a linear DNA (λ-DNA) was also used as a substrate for cleavage. As the methods of plasmid DNA, first we found optimal condition of cleavage reaction through a serious of optimize experiment. As shown in Figure 4, linear DNA was gradually degraded into smears of progressively smaller fragments in the presence of compound **2c**. The efficient cleavage was detected when the concentration of **2c** was just 140 μM.

The UV absorption spectra of the Zn (II) complexes of **2c** and **1** changed significantly as a result of their binding to calf thymus DNA. Monitoring such changes would be helpful in estimating the binding constants. Comparative measurements were carried out with complexes **2c** and **1**. In titration of the Zn (II) complexes (each 50 μM) with ct-DNA (**1**: 0–62.5 μM and **2c**: 0–7.87 μM) in Tris–HCl buffer (40 mM, pH 8.0) at 25 °C. The absorption maxima of **1** (λ_{1max} = 276 and λ_{2max} = 281 nm), and **2c** (λ_{1max} = 276, λ_{2max} = 282 nm) decreased with increasing concentration of DNA (hypochromicity) (Fig. 5a and b). Hypochromism was suggested to be due to strong interactions between the electronic states of the intercalating chromophore and that of the DNA base pairs.[28] However, no apparent isosbestic point was observed in the absorp-

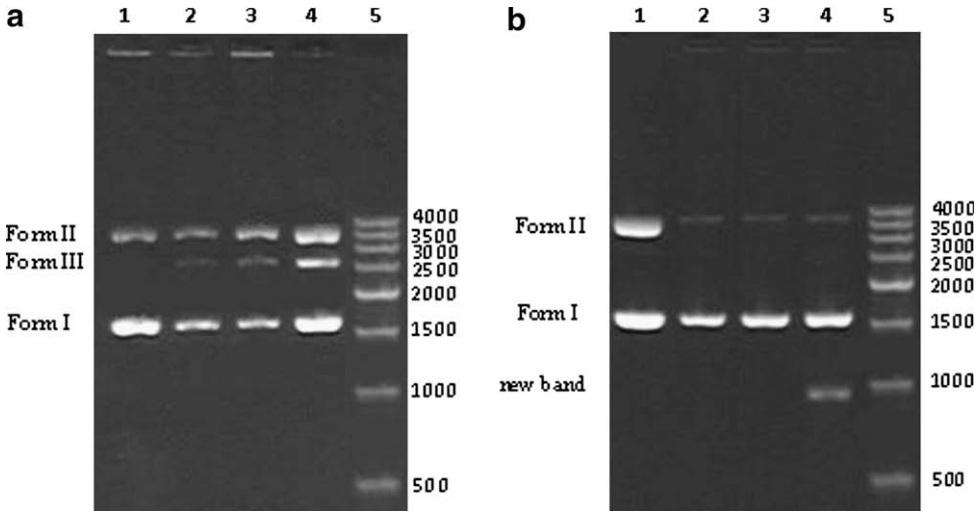

Figure 3. Agarose gel electrophoresis of cleavage reaction of pUC18 DNA (0.08 μg/μl) by different complexes (a: 0.6 mM, b: 2.0 mM) in a Tris–HCl buffer (40 mM, pH 8.92) for 6 h at 50 °C. Lane 1: DNA control; Lane 2–4: **2a**, **2b**, **2c**; Lane 5: 500 bp DNA ladder.

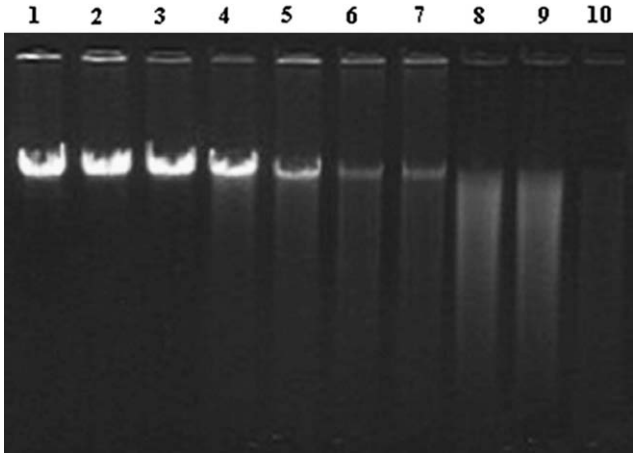

Figure 4. Agarose gel electrophoresis of cleavage reaction of λ-DNA cleaved by complex **2c**. Lane 1: control; Lane 2–10: incubated with **2c** by different concentration (20, 40, 60, 80, 100, 120, 140, 160, 180 μM, respectively) in Tris–HCl buffer (40 mM, pH 8.11) for 6 h at 50 °C.

tion spectra of **2c**. This result suggested that **2c** bound to DNA by multiple modes and not intercalation.[29]

Furthermore, to study further the DNA sequences preference of the Zn (II) complex, the titration experiment was performed in the presence of synthetic double-stranded DNAs (Fig. 5c and d). Among Figure 5b–d, calf thymus DNA and poly[(dA–dT)$_2$] gave similar titration behaviors, except for poly[(dG–dC)$_2$]), where irregular spectrophotometric titration was seen. The phenomenon occurred on account of the different binding affinities to DNAs. In other words, Zn (II) complex with oligopolyamide backbone possessed A-T rich sequences preference. The conclusion was proved further through the K_{app} value (see Table 1).

The smooth decreases in the maximum absorbances allowed us to construct the half-reciprocal plots for [DNA] with matching [Zn (II) complexes] according to the equation (1).[30] The K_{app} determined for the binding of the Zn (II) complexes (**1** and **2c**) and free ligand (**4c**) to calf thymus DNA and synthetic DNAs are summarized in Table 1. In view of the K_{app} values obtained, the complex **1** was calculated to possess nearly the same binding affinities to all the measured DNAs. Without oligopolyamide backbone, the binding activities were very limited for any DNA (K_{app} = 0.78 × 10^4 for calf thymus DNA, 1.61 × 10^4 for poly[(dA–dT)$_2$], and 0.81 × 10^4 for poly[(dG–dC)$_2$]). However, the binding affinities of complex **2c** to calf thymus DNA and poly[(dA–dT)$_2$] were 16-fold and 20.7-fold higher than those for complex **1**, respectively. It showed that the affinity of Zn (II) complex was enhanced on the account of modification with oligopolyamide. The affinities between complex **2c** and all DNAs indicate that the K_{app} value for poly[(dA–dT)$_2$] was approximately 3-fold and 70-fold higher than those for calf thymus DNA and poly[(dG–dC)$_2$]. The outstanding results further supported A-T rich sequences preference of Zn (II) complex in the presence of oligopolyamide. Moreover, we also found that the metal ion played an important role in binding affinity to DNA and the conclusion was confirmed by contrasting data in Table 1. In conclusion, experimental data indicated that introduction of oligopolyamide lead to increasing of binding affinities to A-T rich DNA sequences. Further studies including physicochemical measurements and other techniques will be done to confirm such explanation.

In summary, we synthesized and characterized Zn (II) complexes appended with oligopolyamide backbone. The preliminary biological activity studies showed that the efficient cleavage of plasmid DNA was observed at lower reaction concentration and short time comparing with the complex without oligopolyamide. Interestingly, at a high reaction concentration (1.2 mM), Zn (II) complex converted the plasmid DNA to a new band. Additionally, The UV spectral changes suggested that the strong interactions be-

Table 1
Apparent binding affinity of various complexes to DNA

DNA	Apparent binding constant (K_{app}) (M^{-1}) × 10^{-4}		
	1	**2c**	**4c**
Calf thymus DNA	0.78	12.5	5.00
poly[(dA–dT)$_2$]	1.61	33.3	11.5
poly[(dG–dC)$_2$]	0.81	0.45	–[a]

[a] K_{app} value could not be determined due to irregular spectral changes, see text.

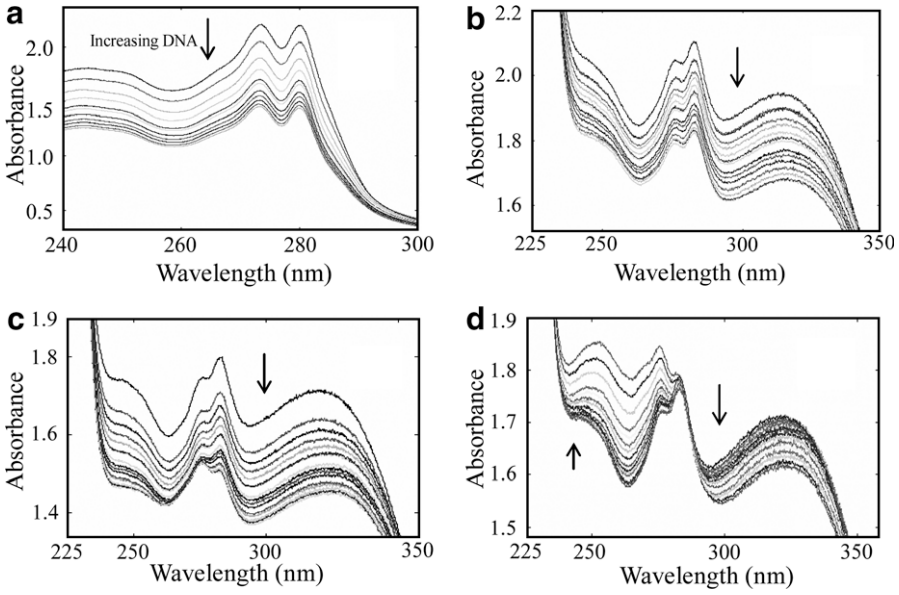

Figure 5. UV spectrophotometric titrations: (a) and (b) for **1** and **2c** (each 50 μM) with calf thymus DNA ((a) 0–62.5 μM and (b) 0–7.87 μM from top to bottom curves); (c) and (d) for **2c** (each 50 μM) with poly[(dA–dT)$_2$] and poly[(dC–dG)$_2$], respectively.

tween DNA and complexes could be observed. Quantification of the binding affinity K_{app} showed that Zn (II) complex with oligopolyamide backbone possessed A-T rich sequences preference apparently.

Acknowledgments

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References and notes

1. Livieri, M.; Mancin, F.; Tonellato, U.; Chin, J. *Chem. Commun.* **2004**, 2862.
2. Rammo, J.; Hettich, R.; Roigk, A.; Schneider, H.-J. *Chem. Commun.* **1996**, 105.
3. Hettich, R.; Schneider, H. J. *J. Am. Chem. Soc.* **1997**, *119*, 5638.
4. Dixon, N. E.; Geue, R. J.; Lambert, J. N.; Moghaddas, S.; Pearce, D. A.; Sargeson, A. L. *Chem. Commun.* **1996**, 1287.
5. Schnaith, L. H.; Hanson, R. S.; Que, L., Jr. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 569.
6. Ragunathan, K. G.; Schneider, H.-J. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1219.
7. Komiyama, M.; Takeda, N.; Shigekawa, H. *Chem. Commun.* **1999**, 1443.
8. Branum, M. E.; Tipton, A. K.; Zhu, S.; Que, L., Jr. *J. Am. Chem. Soc.* **2001**, *123*, 1898.
9. Chand, D. K.; Schneider, H.-J.; Bencini, A.; Bianchi, A.; Giorgi, C.; Ciattini, S.; Valtancoli, B. *Chem. Eur. J.* **2000**, *6*, 4001.
10. Deck, K. M.; Tseng, T. A.; Burstyn, J. N. *Inorg. Chem.* **2002**, *41*, 669.
11. Ren, R.; Yang, P.; Zheng, W.; Hua, Z. *Inorg. Chem.* **2000**, *39*, 5454.
12. Sissi, C.; Mancin, F.; Palumbo, M.; Scrimin, P.; Tecilla, P.; Tonellato, U. *Nucleosides, Nucleotides & Nucleic Acids* **2000**, *19*, 1265.
13. Sreedhara, A.; Freed, J. D.; Cowan, J. A. *J. Am. Chem. Soc.* **2000**, *122*, 8814.
14. Franklin, S. J. *Curr. Opin. Chem. Biol.* **2001**, *5*, 201.
15. Hegg, E. L.; Burstyn, J. N. *Coord. Chem. Rev.* **1998**, *173*, 133.
16. Boseggia, E.; Gatos, M.; Lucatello, L.; Mancin, F.; Moro, S.; Palumbo, M.; Sissi, C.; Tecilla, P.; Tonellato, U.; Zagotto, G. *J. Am. Chem. Soc.* **2004**, *126*, 4543.
17. Basile, L. A.; Raphael, A. L.; Barton, J. K. *J. Am. Chem. Soc.* **1987**, *109*, 7550.
18. Aka, F. N.; Akkaya, M. S.; Akkaya, E. U. *J. Mol. Catal. A* **2001**, *165*, 291.
19. Ichikawa, K.; Tarani, M.; Uddin, M. K.; Nakata, K.; Sato, S. *J. Inorg. Biochem.* **2002**, *91*, 437.
20. Fitzsimons, M. P.; Barton, J. K. *J. Am. Chem. Soc.* **1997**, *119*, 3379.
21. Copeland, K. D.; Fitzsimons, M. P.; Houser, R. P.; Barton, J. K. *Biochemistry* **2002**, *41*, 343.
22. Sissi, C.; Rossi, P.; Felluga, F.; Formaggio, F.; Palumbo, M.; Tecilla, P.; Toniolo, C.; Scrimin, P. *J. Am. Chem. Soc.* **2001**, *123*, 3169.
23. Geierstanger, B. H.; Mrksich, M.; Dervan, P. B.; Wemmer, D. E. *Science* **1994**, *266*, 646.
24. Kielkopf, C. L.; Baird, E. E.; Dervan, P. B.; Rees, D. C. *Nat. Struct. Biol.* **1998**, *5*, 104.
25. White, S.; Daird, E. E.; Dervan, P. B. *Biochemistry* **1996**, *35*, 12532.
26. Jun-hua, X.; Gu, Y. *J. Org. Chem.* **2000**, *65*, 5506.
27. These new compounds were fully characterized. For example, **4c**: ^{1}H NMR (DMSO-d_6, 600 MHz) δ:13.21(m, 1H), 10.24(s, 1H), 9.93(s, 1H), 9.83(s, 1H), 8.17(s, 1H), 7.99(t, 1H), 7.59(d, 5H, J = 1.8), 7.26(d, 1H),7.24(d, 1H), 7.18(q, 4H, J = 9.0), 7.16(d, 1H, J = 1.2), 7.05(q, 2H, J = 7.8), 6.85(d, 1H, J = 1.8), 5.03(s, 2H), 4.88(s, 2H), 3.97(s, 3H), 3.89(s, 3H), 3.85(s, 3H),3.77(s, 3H), 3.17(t, 2H), 2.54(t, 2H), 1.74(t, 2H). ESI-MS: m/z = 881.4[M+H]$^+$. IR (υ_{max}/cm^{-1},KBr disc): 3391, 3132, 2923, 1649, 1584, 1532, 1465, 1435, 1402, 1309, 1253, 1208, 1111, 889, 814, 749, 623, 436. HRMS: found m/z = 881.3596 [M+H]$^+$, $C_{44}H_{45}N_{14}O_7$ requires 881.3601. **2c**: ^{1}H NMR (DMSO-d_6, 600 MHz) δ:10.27(s, 1H), 9.96(s, 1H), 9.85(s, 1H), 8.19(d, 1H, J = 1.8), 8.00(t, 1H, J = 10.8), 7.63(m, 4H), 7.59(d, 1H, J = 1.8), 7.27(d, 1H, J = 1.8), 7.24(d, 1H, J = 1.8), 7.23(m, 4H), 7.16(d, 1H, J = 1.2), 7.05(q, 2H, J = 7.2), 6.85(d, 1H, J = 1.8), 5.07(s, 2H), 4.90(s, 2H), 3.97(s, 3H), 3.87(s, 3H), 3.85(s, 3H), 3.76(s, 3H), 3.14(q, 2H), 2.46(t, 2H), 1.72(t, 2H). ESI-MS: m/z = 943.2[M-2ClO$_4$]$^+$. IR (υ_{max}/cm^{-1},KBr disc): 3266, 3127, 2941, 1644, 1584, 1532, 1463, 1434, 1402, 1309, 1251, 1205, 1110, 1060, 1024, 946, 887, 840, 813, 747, 672, 609, 432.
28. Ying, C.; Xi-wen, H. *Spectrochim. Acta, Part A* **1998**, *54*, 8835.
29. Ishida, K.; Asao, T. *Biochim. Biophys. Acta* **2002**, *1587*, 155.
30. The K_b values were calculated on the basis of the following equation: [DNA]/($\varepsilon_A - \varepsilon_F$) = [DNA]/($\varepsilon_B - \varepsilon_F$) + 1/$K_b$($\varepsilon_B - \varepsilon_F$), where ε_A, ε_F, and ε_B correspond to A/[complex], the molar absorptivity for the free Zn (II) complex, and the molar absorptivity of the Zn (II) complex in the fully bound form, respectively. In plots of [DNA]/($\varepsilon_A - \varepsilon_F$) versus [DNA], K_b is given by the ratio of the slope to the intercept.