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Efficient enhancement of DNA cleavage activity by introducing guanidinium groups into diiron(III) complex

Xiaoqiang Chen,[a] Jingyun Wang,[b,*] Shiguo Sun,[a] Jiangli Fan,[a] Song Wu,[b] Jianfeng Liu,[b] Saijian Ma,[b] Lizhu Zhang[a] and Xiaojun Peng[a,*]

[a]*State Key Laboratory of Fine Chemicals, Dalian University of Technology, Dalian 116012, PR China*
[b]*Department of Bioscience and Biotechnology, Dalian University of Technology, Dalian 116023, PR China*

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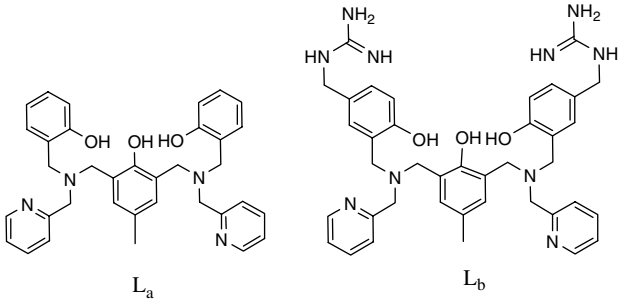
Abstract—Inspired by the structures of natural nucleases, guanidinium groups were introduced into binuclear iron(III) systems. Compared with the corresponding analogue without guanidinium groups, the new diiron(III) system led to considerable rate enhancement on DNA cleavage. The cooperativity between metal ions and guanidine groups was evidenced by the fact that no significant cleavage was observed after incubating pBR322 plasmid DNA with non-metalated ligands or free Fe^{3+} ion. DNA binding experiments indicated that introduction of positively charged guanidinium groups can obtain more than one order of magnitude enhancement in the affinity of complex with DNA.

Mimicking the activities of nucleases is currently an attractive research area in molecular biology since artificial nucleases have potential applications as novel restriction enzymes and anticancer therapeutic agents.[1] In the past few years, various synthetic metallonucleases have been reported as DNA cleavage agents.[2] Furthermore, much effort has focused on di- or polynuclear metal complexes, which are typically more reactive than the corresponding mononuclear metal complexes.[3] Nevertheless, their activities are still much lower than that of the corresponding enzymes. In nature, the high reactivity of nucleases is attributed to the cooperation between metal ions and several functional groups of the amino acid side chains present in the active site. The positively charged residues of lysine, arginine, and histidine are thought to stabilize phosphorane-like transition states by electrostatic interactions, hydrogen bonding, and/or proton transfer.[4] Inspired by the structures of metalloenzymes, several examples of synthetic systems have been reported and exhibited the enhanced activity by introducing ammonium/guanidinium group to monometallic complex.[5] However, no study upon introducing these hydrogen bonding donors to dinuclear metal complex as DNA cleavage agent was reported so far.

2,6-Bis[((2-hydroxybenzyl)(2-pyridylmethyl)amino)methyl]-4-methylphenol (L_a), which provides a N_4O_3-donor coordination sphere, can bind two Fe(III) or Zn(II) ions to form corresponding dinuclear complexes.[6] In this study, we prepared a new dinucleating ligands L_b as described in Scheme 1, whose structure features bisguanidinium-containing arms. In the previous literatures, the complexes based on diiron(III) core have also showed high activity in DNA hydrolysis.[7] We expect that the combination of diferric center and bisguanidinium groups can further enhance DNA cleavage activity.

Beginning with 2-(4-hydroxy-benzyl)-isoindole-1,3-dione, L_b was synthesized according to the reaction



L_a L_b

Scheme 1. Molecular structures of ligands used.

* Corresponding authors. Tel.: +86 411 88993899; fax: +86 411 88993906 (X.P.); e-mail: pengxj@dlut.edu.cn

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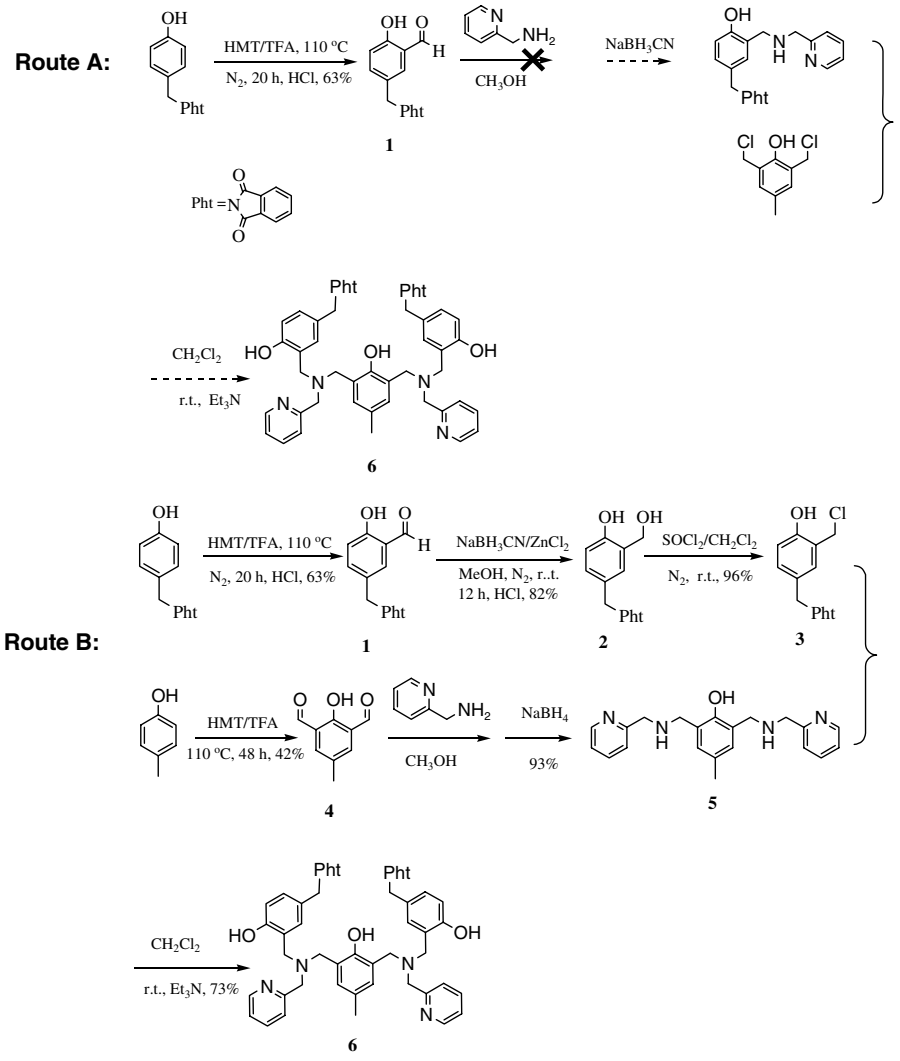
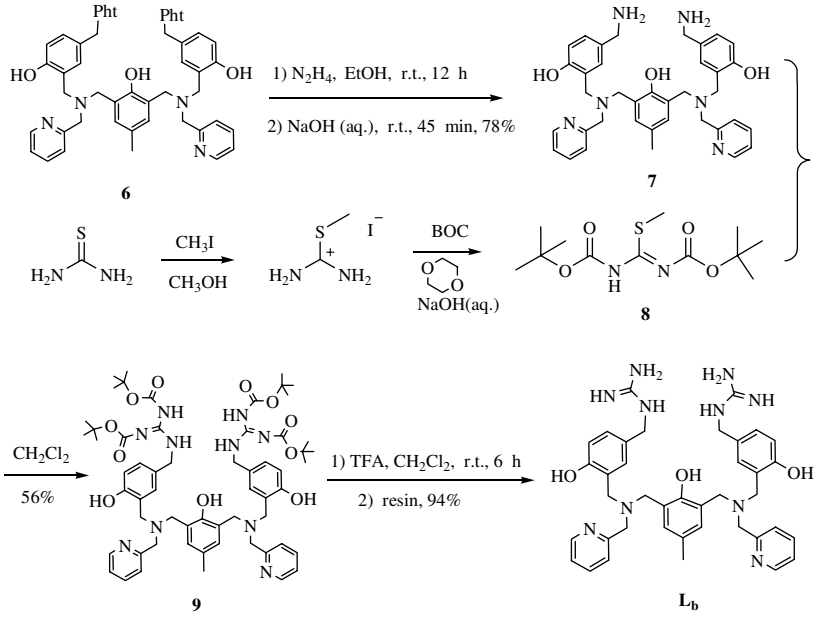


Scheme 2. Synthesis of intermediate **6**.



Scheme 3. Synthesis of L_b.

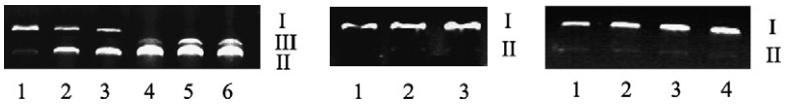


Figure 1. Left: agarose gel electrophoresis of pBR322 plasmid DNA treated with Fe–L_a (lanes 2–3) or Fe–L_b (lanes 4–6) for 1 h in Hepes buffer (20 mM, pH 7.0) at 37 °C. Key: Lane 1, control DNA; Lane 2, Fe–L_a 20 μM. Lane 3, Fe–L_a 50 μM. Lane 4, Fe–L_b 10 μM. Lane 5, Fe–L_b 20 μM. Lane 6: Fe–L_b 50 μM. Middle: agarose gel electrophoresis of pBR322 plasmid DNA treated with ligands for 2 h in Hepes buffer (20 mM, pH 7.0) at 37 °C. Key: Lane, 1 L_a 50 μM. Lane 2, L_b 50 μM. Lane 3, control DNA. Right: agarose gel electrophoresis of pBR322 plasmid DNA treated with $FeCl_3$ for 1 h in Hepes buffer (20 mM, pH 7.0) at 37 °C. Key: Lane 1, control DNA. Lane 2, $FeCl_3$ 20 μM. Lane 3, $FeCl_3$ 40 μM. Lane 4, $FeCl_3$ 100 μM.

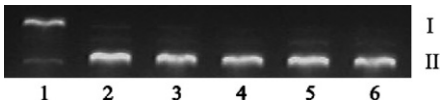


Figure 2. Time-dependence of pBR322 plasmid DNA cleavage by 20 μM Fe–L_b in Hepes buffer (20 mM, pH 7.0) at 37 °C. Key: Lane 1, control. Lane 2, 1 min. Lane 3, 2 min. Lane 4, 4 min. Lane 5, 7 min. Lane 6, 12 min.

sequences depicted in Schemes 2 and 3. To obtain the precursor **6**, we initially sought a similar route (Scheme 2, route A) used previously by Neves and co-workers in the synthesis of L_a.[6b] Unexpectedly, the phthalimide group (Pht) of monoformylated phenol was cleaved under basic conditions due to the presence of pyridin-2-ylmethanamine. Another route (Route B) was chosen to keep phthalimide group from attack of primary amine: first, functionalization in the ortho position of phenol was achieved by formylation via the Duff reaction,[8] and monoformylated phenol was obtained through controling the reaction condition; subsequently, reduction of the monoformylated phenol by $NaBH_3CN$ then gave hydroxymethyl phenol **2**; after chlorination, the resulting chloromethyl phenol **3** was finally incubated with 2-hydroxy-5-methylisophthalaldehyde **5** in the presence of triethylamine to yield the precursor **6**. Deprotection of **6** with hydrazine hydrate in ethanol at room temperature produced **7**. The synthesis of L_b from **7** was preformed according to the similar procedure described by Hassan and coworkers:[9] after guanylation of **7** using *N*,*N*′-bis(*tert*-butoxycarbonyl)-S-methylisothiourea **8**, the Boc-protected molecule **9** was deprotected in the presence of trifluoroacetic acid and then afforded the bisguanidinium ligand L_b[12] by passing through an anion-exchange column.

Stock solutions of complexes (Fe–L_a and Fe–L_b) using in DNA cleavage experiment were prepared through incubating ligands with 2-equiv $FeCl_3 6H_2O$ in water. Through HRMS spectra, the binuclear structures of complexes were supported by the information that the measured isotopic distribution is coincident with the calculated one (Figure S1 and S2). Incubation of pBR322 plasmid DNA (0.02 μg/μL, 32 μM bp) with the stock solution of complexes at pH 7.0 and 37 °C for 1 h results in a different extent of DNA cleavage depending on the nature and concentration of the complexes (Fig. 1, left)[13]. Complex Fe–L_a, lacking guanidinium subunit, shows a much lesser cleavage activity: after incubation of DNA in the presence of 50 μM of Fe–L_a, only about 66% of form I have been nicked. However, for Fe–L_b, just 10 μM was required to convert completely from the supercoiled form I to the nicked form II and eventually to the linearized form III. This result indicates a much more pronounced DNA cleavage efficiency for the introduction of bisguanidinium groups. The cooperativity between metal ions and guanidine groups is evidenced by the fact that no significant cleavage was observed after incubating pBR322 plasmid DNA with 50 μM non-metalated ligands for 2 h (Fig. 1, middle) or with 20–100 μM $FeCl_3$ for 1 h (Fig. 1, right). Furthermore, we carried out kinetic measurements of the DNA cleavage reaction by Fe–L_b. As indicated in Fig. 2, 20 μM Fe–L_b led to complete cleavage of form I in a very short time (no more than 1 min), then remains active at a much slower rate. By the reason of too high ini-

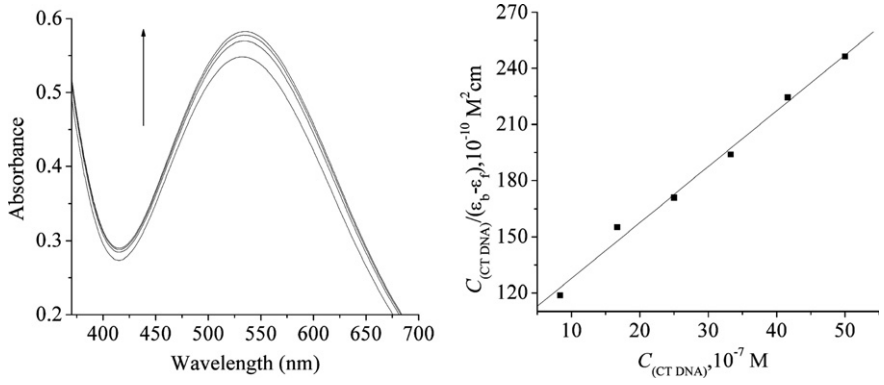
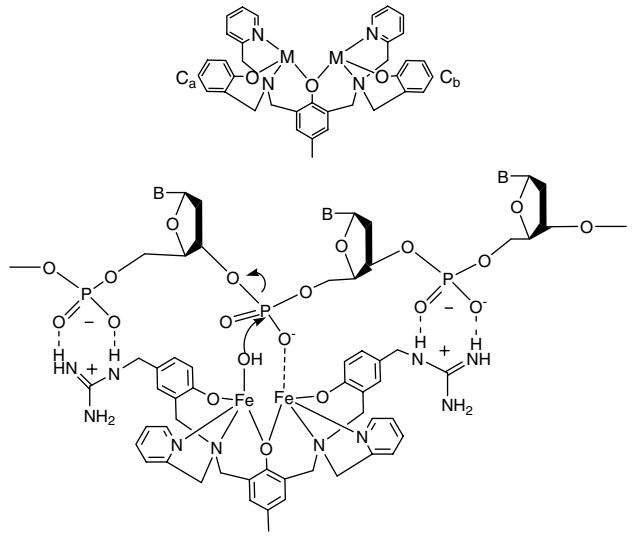


Figure 3. Left: absorption spectra of Fe–L_b (2×10^{-4} M) in the presence of increasing amounts of CT DNA at room temperature. Right: the plot of $C_{DNA}/(\varepsilon_a - \varepsilon_f)$ versus C_{DNA}.

tial activity, attempt to obtain the first-order rate constant of DNA cleavage is unsuccessful.

To exploring the interaction between complexes and DNA, we carried out absorption titration measurements[14]. With increasing concentration of CT DNA, the changes of the intensity of the spectral band at 531 nm for Fe–L_a and 526 nm for Fe–L_b were monitored. The binding constant was determined using the following equation:[10] $C_{DNA}/(\varepsilon_a - \varepsilon_f) = C_{DNA}/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_b - \varepsilon_f)$, where ε_a, ε_f, and ε_b correspond to A_{obsd}/[complex], the extinction coefficient for the free iron complex, and the extinction coefficient for the iron complex in the fully bound form, respectively. The intrinsic binding constants of complexes were obtained by the ratio of the slope to intercept through the plot of $C_{DNA}/(\varepsilon_a - \varepsilon_f)$ versus C_{DNA}: $1.9 \times 10^4\ M^{-1}$ for Fe–L_a and $3.0 \times 10^5\ M^{-1}$ for Fe–L_b (Fig. 3). Comparing with that of Fe–L_a, more than one order of magnitude enhancement in binding abilities of Fe–L_b should be attributed to the more positive charges and hydrogen bonding effects of bisguanidinium groups. This result also indicated that the introduction of guanidinium groups could potentially accelate DNA cleavage by increasing the DNA affinity of complex with DNA.

A possible mechanism (Scheme 4) for the cleavage of DNA by Fe–L_b was proposed. From the crystal structure data of complexes based on L_a, the distance between C_a and C_b is calculated to be about 12 Å (Scheme 4, the top graphic).[6b,c] On the other hand, since the distance between two consecutive phosphate groups in a B-DNA strand is about 7 Å,[11] we may conceive a likely model of interaction between Fe–L_b and DNA: the two guanidinium groups interact with the adjacent phosphate groups, and such an arrangement forces the central phosphate to interact with the two metal centers, taking full advantage of their complementary roles for the hydrolytic cleavage (Scheme 4, the bottom graphic).



Scheme 4. Top: stuctures of complexes based on L_a (M = Fe or Zn); Bottom: proposed mechanism for cleavage of DNA by Fe–L_b.

In summary, guanidinium groups have been introduced successfully to dinuclear iron(III) system. Compared with the analogue (Fe–L_a), the new bimetallic system led to considerable rate enhancement. The cooperative action between the guanidinium groups and the two metal centers is evidenced by the fact that there is no significant cleavage using non-metalated ligands or $FeCl_3$. Absorption titration experiments indicate that introducing of positively charged guanidinium groups to diiron(III) complex can lead to more than one order of magnitude enhancement in DNA binding abilities.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.11.001.

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12. Ligand L_b: ^{1}H NMR (CD_3OD, 400 MHz, δ ppm): 8.59 (d, J = 6.0 Hz, 2H, Py–H), 8.07 (br, 2H, Py–H), 7.77 (d, J = 6.0 Hz, 2H, Py–H), 7.60 (s, 2H, Ph–H), 7.36 (br, 2H, Py–H), 7.35 (s, 2H, Ph–H), 7.15 (d, J = 8.0 Hz, 2H, Ph–H), 6.72 (d, J = 8.0 Hz, 2H, Ph–H), 4.72 (s, 4H, $-CH_2N-$), 4.62 (s, 4H, $PyCH_2-$), 4.50 (s, 4H, $-CH_2N-$), 4.27 (s, 4H, $-CH_2N-$), 2.64 (s, 3H, Ph–CH_3); ^{13}C ($CDCl_3$, 100 MHz) δ 158.49, 157.15, 154.55, 149.04, 147.16, 143.10, 136.66, 132.87, 132.80, 132.20, 129.25, 127.90, 127.03, 120.22, 117.82, 116.64, 56.44, 56.40,56.36, 45.23, 20.42; HRMS calcd for $C_{39}H_{46}N_{10}O_3$, $[M+H]^+$, 704.3911. Found: 704.3924.
13. *pBR322 DNA cleavage experiments by binuclear metal complexes:* DNA cleavage experiments were performed using pBR 322 (Takara) in 20 mM Hepes buffer, pH 7.0, with incubating DNA (0.4 μg) at 37 °C in the presence and absence of metal complex for the definite time. The cleavage reactions were stopped by addition of 3 μL of loading buffer (0.25% bromophenol blue, 25% glycerol, 1 mM EDTA, 2% SDS). The electrophoresis of DNA cleavage products was performed on 1% agarose gel. The gels were run at 130 V for 45 min in 0.01 M, pH 7.0, TAE buffer. The resolved bands were visualized by ethidium bromide staining and quantified. A correction factor of 1.22 was utilized to account for the decrease in ability of ethidium bromide to intercalate into form I DNA versus forms II and III.
14. *Absorption titration of complexes binding to DNA:* The solutions of CT DNA gave a ratio of UV absorbance at 260 and 280 nm, $A_{260}/A_{280} > 1.8$, indicating that the DNA was sufficiently free of protein. The concentrated stock solution of CT DNA (stored at 4 °C and used not more than 6 days) was prepared, and the concentration of DNA in nucleotide phosphate was determined by UV absorbance at 260 nm. The molar absorption coefficient of CT DNA was taken as 6600 $M^{-1}cm^{-1}$. A solution (3 ml) of complexes in water was incubated for 30 min at 25 °C. With addition of increasing amounts of DNA, the influence of volume was negligible.