Bioorganic & Medicinal Chemistry Letters 19 (2009) 4139–4142

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

# Bisintercalator-containing dinuclear iron(III) complex: An efficient artificial nuclease

Xiaoqiang Chen [a], Jiangli Fan [a], Xiaojun Peng [a,*], Jingyun Wang [b,*], Shiguo Sun [a], Rong Zhang [a], Tong Wu [a], Fei Zhang [a], Jianfeng Liu [b], Fen Wang [b], Saijian Ma [b]

[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

ARTICLE INFO

*Article history:*
Received 8 February 2009
Revised 29 May 2009
Accepted 1 June 2009
Available online 6 June 2009

*Keywords:*
Bisintercalator
Dinuclear Iron(III) complex
Artificial nuclease

ABSTRACT

Two acridine groups were successfully introduced into di-iron(III) complex. DNA cleavage experiments indicated that complex conjugating bisacridine groups can enhance 300-fold for the cleavage efficiency compared with complex lacking of acridine conjugation. Further ligation assay of DNA segments provided the evidence for hydrolytic mechanism of DNA cleavage.



Oxidative and hydrolytic artificial nucleases have been attracting increasing attention in the field of genomics and biotechnologies due to their potential applications in molecular biology and therapy.[1] Oxidative cleavage requires co-reactants to initiate cleavage, and the cleavage is mediated by reactive oxygen species (ROS) that cause other severe cytotoxic effects.[2] As a consequence, pathways that result in DNA cleavage by a hydrolysis mechanism are preferable. However, the phosphodiester bonds of DNA are exceptionally resistant to hydrolysis. Natural nucleases such as restriction endonucleases and topoisomerases can efficiently catalyze DNA hydrolysis, which attribute to the active metallic center.[1a] In recent years, various complexes including mono-, di-, and polynuclear have been reported as DNA hydrolytic cleavage agents.[3] Di- or polynuclear complexes are typically more reactive than the corresponding monometallic complexes.[4] Nevertheless, their activities are still much lower than that of the natural nucleases.

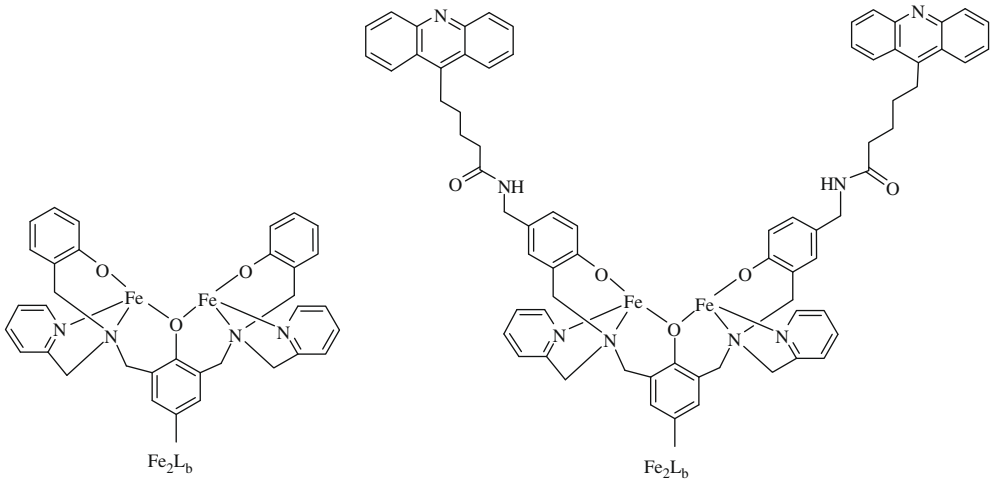
The reactivity of DNA cleaving systems can be enhanced by conjugating with high DNA-affinity group. But surprisingly, examples of metal complexes appended to intercalating groups as hydrolytic agents are rare.[3c,5] In 2004, Tecilla et al. synthesized a series of Zn(II) complex-anthraquinone intercalator conjugates, and the reactivity studies showed that the length of the spacer which tethers the intercalating unit to the catalytic group was a key element for the cleavage activity. In the case of flexible spacer, the introduction of anthraquinone group can lead to a 15-fold increase of the cleavage efficiency of DNA.[3c] Our group reported previously a diiron(III) system bearing single-acridinium group, and DNA cleavage experiment showed that the conjugation of the diiron(III) complex with single-acridinium group can lead to a 14-fold increase the hydrolytic cleavage efficiency when compared with the complex lacking the intercalating moiety.[5e] In addition, in the previous literatures, bisintercalators showed more excellent abilities in DNA binding and sequence specificity than the corresponding compound with single intercalator,[6] but no study upon introducing bisintercalator to dinuclear metal complex as DNA hydrolytic agent was reported so far.

Fe(III) ion most frequently occurs in natural nucleases, but unlike Fe(II) and Zn(II), it has been scarcely employed for artificial nucleases, and only very few Fe(III) complexes with hydrolytic activity in DNA cleavage are reported in the literature so far.[3f,g,5e] 2,6-Bis[((2-hydroxybenzyl)(2-pyridylmethyl)amino)methyl]-4-methylphenol ($\mathbf{L_a}$), which provides a $N_4O_3$-donor coordination sphere, can bond two Fe(III) or Zn(II) ions to form corresponding dinuclear complexes.[7] In this Letter, we reported a new dinucleating complex $\mathbf{Fe_2L_b}$, whose structure bearing bisacridine-containing arms (Scheme 1). We expect the combination of diferric centers and bisacridine moieties can further enhance DNA cleavage activity. The long alkyl chain between intercalator and dinuclear moiety was arranged since too short spacer is likely to disturb the reactivity of dinuclear complex.

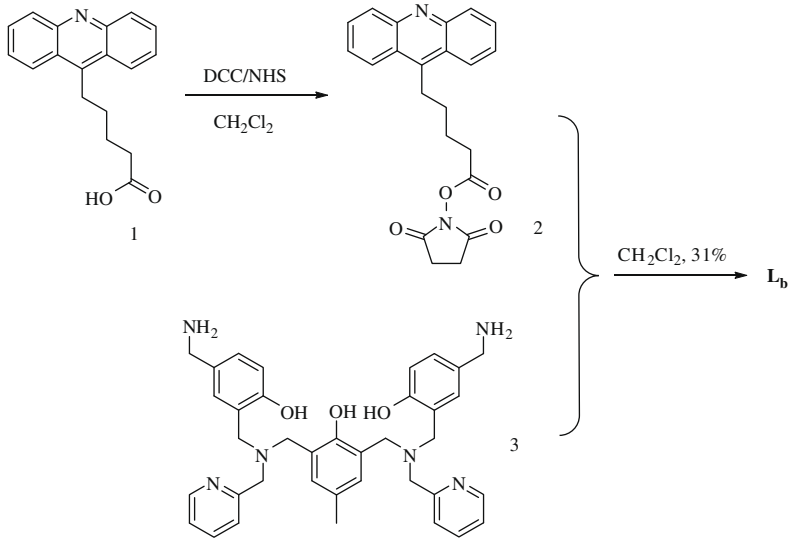
$\mathbf{L_b}$ was synthesized according to the reaction sequence depicted in Scheme 2. 5-(Acridin-9-yl)pentanoic acid, *N*,*N*′-dicyclohexylcarbodiimide (DCC) and *N*-hydroxysuccinimide (NHS) were mixed and stirred for 6 h at room temperature. After purifying by flash column chromatography, the resulting activated ester **2** was made to

* Corresponding authors. Tel.: +86 411 39893899; fax: +86 411 39893906 (X.P.).
*E-mail addresses:* pengxj@dlut.edu.cn (X. Peng), wangjingyun@dl.cn (J. Wang).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.06.003




**Scheme 1.** Molecular structures of complexes used.




**Scheme 2.** The synthesis of $\mathbf{L_b}$.

react with **3** (2,2′-(2-hydroxy-5-methyl-1,3-phenylene)bis(methylene)bis((pyrid-in-2-ylmethyl)azanediyl)bis(methylene)bis(4-(amino methyl)phenol))[8] in $CH_2Cl_2$ for two days to give $\mathbf{L_b}$ in 31% yield. Stock solutions (1 mM) of complexes ($\mathbf{Fe_2L_a}$ and $\mathbf{Fe_2L_b}$) used for DNA binding and cleavage experiments were prepared by incubating ligands with 2 equiv $FeCl_3{\cdot}6H_2O$ in water (for $\mathbf{Fe_2L_a}$) or $H_2O$/$CH_3OH$ (90:10) (for $\mathbf{Fe_2L_b}$) at 50 °C for 1 h. Through HRMS spectra, the binuclear structures of complexes were supported by the measured isotopic distributions which are in agreement with those calculated (Figs. S1 and S2). For $\mathbf{Fe_2L_b}$, the observed species at *m/z* (*z* = 3) = 422.4721 (calcd 422.4748) corresponds to the trivalent cationic diiron species $[Fe(III)_2(L_b{-}3H)(H_2O)]^{3+}$: The six positive charges due to the two $Fe^{3+}$ ions and the three negative charges due to three phenolates ($L_b$–3H) result in a three positively charged species (Fig. 1). Similarly, other peaks can also be attributed to the species with diiron(III) core (Fig. S2).

In the absence of any external agent, incubation of pBR322 plasmid DNA (31 μM bp) with the complexes $\mathbf{Fe_2L_a}$ and $\mathbf{Fe_2L_b}$ at pH 7.0 and 37 °C for 1 h result in a different extent of DNA cleavage depending on the nature and concentration of the complex (Fig. 2). Complex $\mathbf{Fe_2L_a}$, lacking the acridine subunit, shows a far less cleavage activity. After incubation of DNA in the presence of 10 μM of $\mathbf{Fe_2L_a}$, only about 27% of form I was nicked (lane 2). In contrast, for $\mathbf{Fe_2L_b}$, nearly complete transform from the supercoiled form I to nicked form II was found at the same concentration (lane 6). This observation provided a clear evidence for the key role of acridine group in promoting the cleavage reaction rate of diiron(III) catalyst. Figure 3 showed the effect of metal concentration on the cleavage activities. Only when the amount of $Fe^{3+}$ increased twice that of $\mathbf{L_b}$, the activity of system reached maximum. The role of diiron(III) center in $\mathbf{Fe_2L_b}$ for DNA cleavage efficiency was supported by the result.

To better define the behavior of catalyst, we carried out kinetic measurements of the DNA cleavage reaction in the presence of $\mathbf{Fe_2L_a}$ and $\mathbf{Fe_2L_b}$, respectively. The fitting of the kinetic data with first-order decay model show the plasmid DNA was cleaved by 10 μM $\mathbf{Fe_2L_b}$ with the rate constant of $6.6 \times 10^{-3}\ s^{-1}$ (Fig. S3). At the same concentration, the rate constants of supercoiled form I cleavage by $\mathbf{Fe_2L_a}$ was $2.3 \times 10^{-5}\ s^{-1}$ (Fig. S4). Therefore, a 300-fold acceleration in the reaction rate was achieved by introducing bisacridine subunits.

On the other hand, the ligation experiment of cleaved pUC19 DNA also provided the evidence for hydrolytic mechanism (Fig. 4). Incubating pUC19 DNA (123 μM bp) with $\mathbf{Fe_2L_b}$ (30 μM)

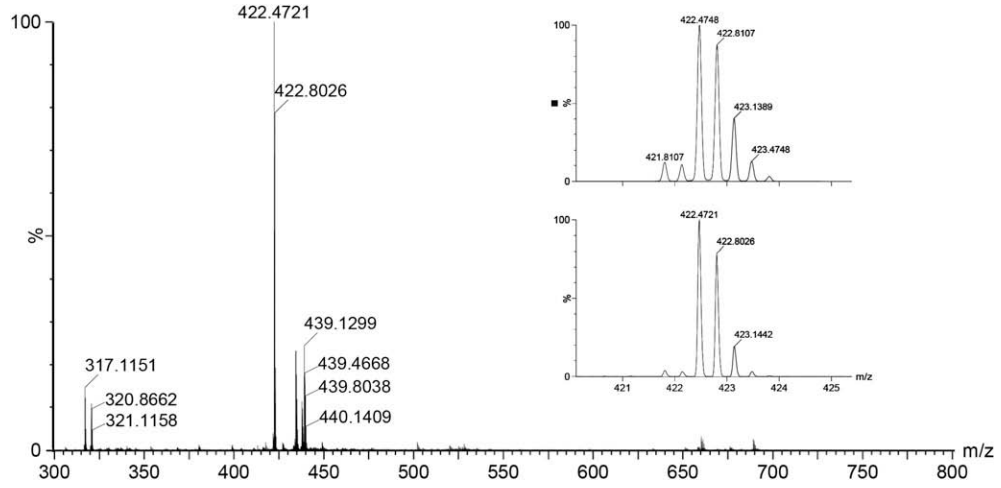



**Figure 1.** High-resolution ESI-MS spectrum of $\mathbf{Fe_2L_b}$: $[Fe(III)Fe(III)(L_b{-}3H)(H_2O)]^{3+}$ 422.4721 (calcd: 422.4748); insert: the calculated isotopic distribution (upper) versus the measured isotopic distribution (lower).

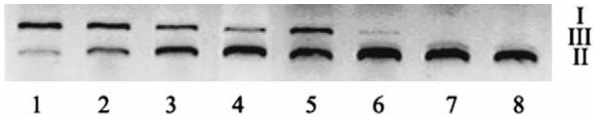



**Figure 2.** Agarose gel electrophoresis of pBR322 plasmid DNA treated with $\mathbf{Fe_2L_a}$ (lanes 2–4) and $\mathbf{Fe_2L_b}$ (lanes 5–8) for 1 h in HEPES buffer (20 mM, pH 7.0) at 37 °C. Key: lane 1, control DNA; lane 2, $\mathbf{Fe_2L_a}$ 10 μM; lane 3, $\mathbf{Fe_2L_a}$ 20 μM; lane 4, $\mathbf{Fe_2L_a}$ 50 μM; lane 5, $\mathbf{Fe_2L_b}$ 5 μM; lane 6, $\mathbf{Fe_2L_b}$ 10 μM; lane 7, $\mathbf{Fe_2L_b}$ 20 μM; lane 8, $\mathbf{Fe_2L_b}$ 50 μM.

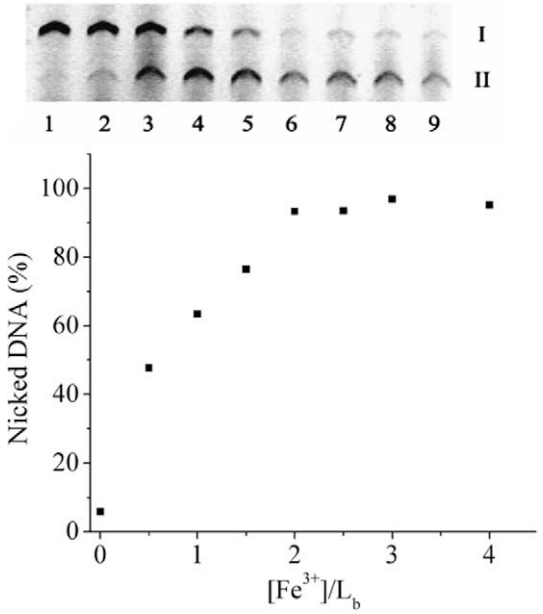



**Figure 3.** Top: agarose gel electrophoresis of pBR322 plasmid DNA treated with fixed concentration of $\mathbf{L_b}$ (10 μM) in the presence of increasing aliquots of $Fe^{3+}$ for 1 h in HEPES buffer (20 mM, pH 7.0) at 37 °C. Key: lane 1, control DNA; lane 2, $Fe^{3+}$ 0 μM; lane 3, $Fe^{3+}$ 5 μM; lane 4, $Fe^{3+}$ 10 μM; lane 5, $Fe^{3+}$ 15 μM; lane 6, $Fe^{3+}$ 20 μM; lane 7, $Fe^{3+}$ 25 μM; lane 8, $Fe^{3+}$ 30 μM; lane 9, $Fe^{3+}$ 40 μM. Bottom: percentage of nicked DNA with fixed concentration of $\mathbf{L_b}$ and increasing amount of $Fe^{3+}$.

for 20 h at 37 °C, the cleaved DNA fragments were mostly distributed over the range of 100 bp to 400 bp (lane 2), which means cleavage with sequence selectivity against DNA to some extent,

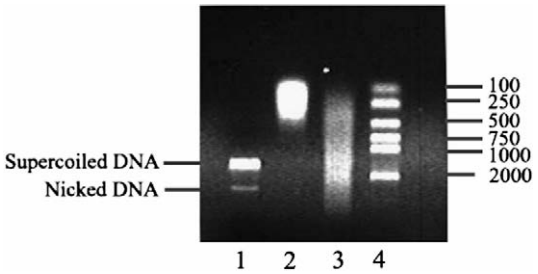



**Figure 4.** Agarose gel electrophoresis of cleaved pUC19 plasmid DNA by $\mathbf{Fe_2L_b}$ following T4 ligase treatment. Key: lane 1, control DNA; lane 2, cleaved pUC19 plasmid DNA by $\mathbf{Fe_2L_b}$; lane 3, cleaved pUC19 plasmid DNA by $\mathbf{Fe_2L_b}$ following T4 ligase treatment; lane 4, D 2000 markers.

otherwise, the cleaved DNA fraction should be observed as smears in electrophoretic gel. After recycled and incubated with T4 ligase for 16 h at 16 °C, most of DNA segments were re-ligated successfully and formed longer DNA molecules. (Fig. 4, lane 3). The results clearly exhibit DNA was cleaved by $\mathbf{Fe_2L_b}$ via hydrolytic pathway, also suggested that the cleavage generated the segments with 5′-phosphate and 3′-hydroxyl termini.

The binding of intercalative complex $\mathbf{Fe_2L_b}$ to DNA has been characterized classically through absorption spectral titration by following the change in absorbance intensity and wavelength. Figure 5 showed the change in the absorption spectra of $\mathbf{Fe_2L_b}$ with increasing DNA concentration. The addition of CT DNA to a solution of complex results in decrease in the molar absorptivity (hypochromism) as well as slight red shift, indicating the binding between acridine groups and DNA. The intrinsic binding constant $K_b$ has been determined from the spectral titration data using the equation[9]: $C_{DNA}/(\varepsilon_a - \varepsilon_f) = C_{DNA}/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_b - \varepsilon_f)$, and $K_b$ was as given $7.6 \times 10^5\ M^{-1}$ by the ratio of slope to intercept.

In summary, bisacridine groups were introduced into diiron(III) system successfully. Absorbance titration results exhibited the new system has high DNA-affinity with the binding constant of $7.6 \times 10^5\ M^{-1}$. DNA cleavage studies showed the introduction of two armed intercalator can lead to a 300-fold enhancement of activity comparing with acridine-lacking complex $\mathbf{Fe_2L_a}$. Furthermore, ligation experiment provided the evidence for hydrolytic cleavage of DNA by $\mathbf{Fe_2L_b}$. The activity versus $[Fe^{3+}]/[L_b]$ revealed the di-Fe(III) center played key role for DNA cleavage.

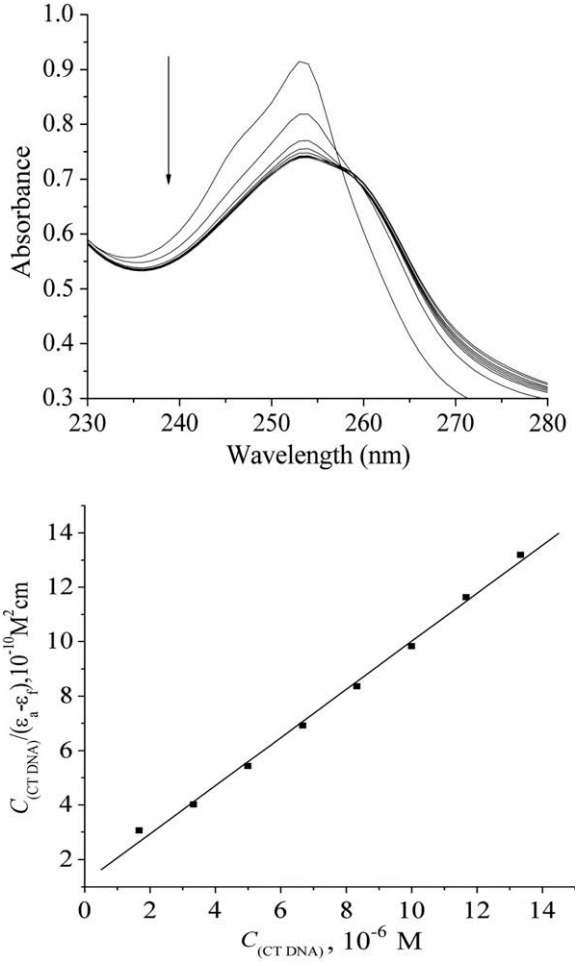



**Figure 5.** Top: absorption spectra of $\mathbf{Fe_2L_b}$ ($1.67 \times 10^{-5}$ M) in the presence of increasing amounts of CT DNA at room temperature. Bottom: the plot of $C_{DNA}/(\varepsilon_a - \varepsilon_f)$ versus $C_{DNA}$. ($K_b = 7.6 \times 10^5\ M^{-1}$).

## Acknowledgements

This work was supported by the Ministry of Education of China (No. 707016) and National Natural Science Foundation of China (Nos. 20706008 and 20725621). We also thank Dr. K. M. K. Swamy for English polishing.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.06.003.

## References and notes

1. (a) Hegg, E. L.; Burstyn, J. N. *Coord. Chem. Rev.* **1998**, *173*, 133; (b) Komiyama, M.; Sumaoka, J. *Curr. Opin. Chem. Biol.* **1998**, *2*, 751; (c) Cowan, J. A. *Curr. Opin. Chem. Biol.* **2001**, *5*, 634; (d) Morrow, J. R.; Iranzo, O. *Curr. Opin. Chem. Biol.* **2004**, *8*, 192; (e) Mancin, F.; Scrimin, P.; Tecilla, P.; Tonellato, U. *Chem. Commun.* **2005**, 2540; (f) Niittymäki, T.; Lönnberg, H. *Org. Biomol. Chem.* **2006**, *4*, 15.
2. (a) Pogozelski, W. K.; McNeese, T. J.; Tuilius, T. D. *J. Am. Chem. Soc.* **1995**, *117*, 6428; (b) Sigman, D. S.; Bruice, T. W.; Mazumder, A.; Sutton, C. L. *Acc. Chem. Res.* **1993**, *26*, 98; (c) Meunier, B. *Chem. Rev.* **1992**, *92*, 1411; (d) Pratviel, G.; Duarte, V.; Bernadou, J.; Meunier, B. *J. Am. Chem. Soc.* **1993**, *115*, 7939.
3. (a) Sreedhara, A.; Freed, J. D.; Cowan, J. A. *J. Am. Chem. Soc.* **2000**, *122*, 8814; (b) Ren, R.; Yang, P.; Zheng, W.; Hua, Z. *Inorg. Chem.* **2000**, *39*, 5454; (c) 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; (d) Matsuda, S.; Ishikubo, A.; Kuzuya, A.; Yashiro, M.; Komiyama, M. *Angew. Chem., Int. Ed.* **1998**, *37*, 3284; (e) Sissi, C.; Rossi, P.; Felluga, F.; Formaggio, F.; Palumbo, M.; Tecilla, P.; Toniolo, C.; Scrimin, P. *J. Am. Chem. Soc.* **2001**, *123*, 3169; (f) Schnaith, L. M. T.; Hanson, R. S.; Que, L., Jr. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 569; (g) Liu, C.; Yu, S.; Li, D.; Liao, Z.; Sun, X.; Xu, H. *Inorg. Chem.* **2002**, *41*, 913; (h) Neves, A.; Terenzi, H.; Horner, R.; Horn, A., Jr.; Szpoganicz, B.; Sugai, J. *Inorg. Chem. Commun.* **2001**, 388; (i) Komiyama, M.; Arishima, H.; Yokoyama, M.; Kitamura, Y.; Yamamoto, Y. *ChemBioChem* **2005**, *6*, 192; (j) Branum, M. E.; Tipton, A. K.; Zhu, S.; Que, L., Jr. *J. Am. Chem. Soc.* **2001**, *123*, 1898; (k) Sumaoka, J.; Azuma, Y.; Komiyama, M. *Chem. Eur. J.* **1998**, *4*, 205; (l) Sheng, X.; Guo, X.; Lu, X.-M.; Lu, G.-Y.; Shao, Y.; Liu, F.; Xu, Q. *Bioconjugate Chem.* **2008**, *19*, 490.
4. (a) Chin, J. *Curr. Opin. Chem. Biol.* **1997**, *1*, 514; (b) Rossi, P.; Felluga, F.; Tecilla, P.; Formaggio, F.; Crisma, M.; Toniolo, C.; Scrimin, P. *J. Am. Chem. Soc.* **1999**, *121*, 6948; (c) Iranzo, O.; Kovalevsky, A. Y.; Morrow, J. R.; Richard, J. P. *J. Am. Chem. Soc.* **2003**, *125*, 1988.
5. (a) Fitzsimons, M. P.; Barton, J. K. *J. Am. Chem. Soc.* **1997**, *119*, 3379; (b) Copeland, K. D.; Fitzsimons, M. P.; Houser, R. P.; Barton, J. K. *Biochemistry* **2002**, *41*, 343; (c) Hashimoto, S.; Nakamura, Y. *Chem. Commun.* **1995**, 1413; (d) Hashimoto, S.; Nakamura, Y. *J. Chem. Soc., Perkin Trans. 1* **1996**, 2623; (e) Chen, X.-Q.; Peng, X.-J.; Wang, J.-Y.; Wang, Y.; Zhang, L.-Z.; Wu, T.; Fan, J.-L.; Wu, Y.-K. *Eur. J. Inorg. Chem.* **2007**, 5400; (f) Tseng, T.-S. A.; Burstyn, J. N. *Chem. Commun.* **2008**, 6209; (g) Chen, X. Q.; Wang, J. Y.; Zhang, T. Y.; Zhang, L. Z.; Peng, X. J. *Chinese Chem. Lett.* **2008**, *19*, 342.
6. (a) Fechter, E. J.; Olenyuk, B.; Dervan, P. B. *Angew. Chem., Int. Ed.* **2004**, *43*, 3591; (b) Chu, Y.; Sorey, S.; Hoffman, D. W.; Iverson, B. L. *J. Am. Chem. Soc.* **2007**, *129*, 1304.
7. (a) Krebs, B.; Schepers, K.; Bremer, B.; Henkel, G.; Althaus, E.; Mueller-Warmuth, W.; Griesar, K.; Haase, W. *Inorg. Chem.* **1994**, *33*, 1907; (b) Neves, A.; de Brito, M. A.; Vencato, I.; Drago, V.; Griesar, K.; Haase, W. *Inorg. Chem.* **1996**, *35*, 2360; (c) Adams, H.; Bradshaw, D.; Fenton, D. E. *Eur. J. Inorg. Chem.* **2002**, 535.
8. Chen, X.; Wang, J.; Sun, S.; Fan, J.; Wu, S.; Liu, J.; Ma, S.; Zhang, L.; Peng, X. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 109.
9. Wolfe, A.; Shimer, G. H.; Meehan, T., Jr. *Biochemistry* **1987**, *26*, 6392.