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Ferrocene-bridging dinuclear cyclen copper(II) complexes as high efficient artificial nucleases: design, synthesis and interaction with DNA

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Two novel cyclen copper(II) complexes bridged by ferrocene were designed and synthesized. Both of these complexes exhibited excellent cleavage ability towards plasmid DNA via an oxidative pathway without the presence of any additives. Cyclic voltammetry was used to investigate the electrochemistry characters of the interaction between the complexes and DNA. Agarose gel electrophoresis was carried out to study the DNA restriction ability of these complexes, and the results indicated that the complexes showed higher cleavage efficiency via an oxidative pathway without the presence of any additives. The mechanism of DNA cleavage catalyzed by these complexes was examined by the addition of various scavengers, and the results showed that singlet oxygen and hydroxyl radical might be responsible for the cleavage process. **Copyright © 2008 John Wiley & Sons, Ltd.**

Keywords: cyclen; copper complex; ferrocene; DNA cleavage

Introduction

The design of highly efficient artificial nucleases is regarded as a great challenge for both chemists and biologists due to their utility as biological tools and as chemotherapeutic agents.[1–4] DNA can be cleaved through hydrolytic, oxidative and photo-induced pathways.[5–7] Among these methods, however, oxidative DNA cleavage has attracted much attention due to its simple procedure and excellent activity.[6,8] A number of transition metal complexes have been reported to be excellent DNA cleavage agents, such as $[Fe(EDTA)]^{2-}$, $[Cu(OP)_2]^{+}$, Fe-BLM, metalloporphyrins, Ni-peptides and metal–salen complexes [for abbreviation, $[Fe(EDTA)]^{2-}$, EDTA = ethylenediaminetetraacetic acid, $[Cu(OP)_2]^{+}$, OP = 1,10-phen-anthroline, Fe-BLM, BLM = bleomycin, metal salen, salen = *N*,*N*-ethylenebis (salicylidene aminato)].[9–14] These complexes could attack the saccharide or base moieties of DNA via an oxidative path.[8] For their biologically accessible redox potential and relatively high affinity to nucleobase,[6] copper complexes have been explored widely as chemical nucleases. Moreover, multi-nuclear complexes have attracted more interest in this field due to their potential cooperative effects between the metal centers.[15,16] The metal centers in multi-nuclear complexes might activate the bound O_2 efficiently, and they could also bind to particular conformations of nucleic acid selectively.[16] On the other hand, multi-metal centers could enhance the electrostatic interaction between the complexes and anionic DNA phosphate backbone.[16]

Simple molecular structure, excellent cleavage activity and the absence of additives are regarded as requirements for a good artificial nuclease. Therefore, finding excellent artificial nucleases that bear simple organic ligands and can cleave DNA via a 'self-activating' process is of great importance.[4,17] In our group, several mono- and di-nuclear cyclen Cu(II), Co(II), Ni(II) and Zn(II) complexes and their applications on the interaction with plasmid DNA have been studied. The influence of the bridge, which could be flexible or rigid in the di-nuclear complexes, was also investigated.[18] Owing to its stability, easy preparation and tunable redox properties, ferrocene and its derivatives, including amino acids, peptides, proteins and DNA conjugates, have been widely used in DNA hybridation detection, chemical sensing, asymmetric catalysis and material science.[19] Moreover, some ferrocene derivatives have been proved to display DNA damage activity and tumor cell growth inhibition.[20] In these complexes, we introduced ferrocene as a bridge to di-nuclear cyclen Cu(II) complexes, and we hoped that this complex would act as a 'self-activating' artificial nuclease with high efficiency.

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Results and Discussion

Preparation of the ferrocene-bridging dinuclear cyclen copper(II) complexes

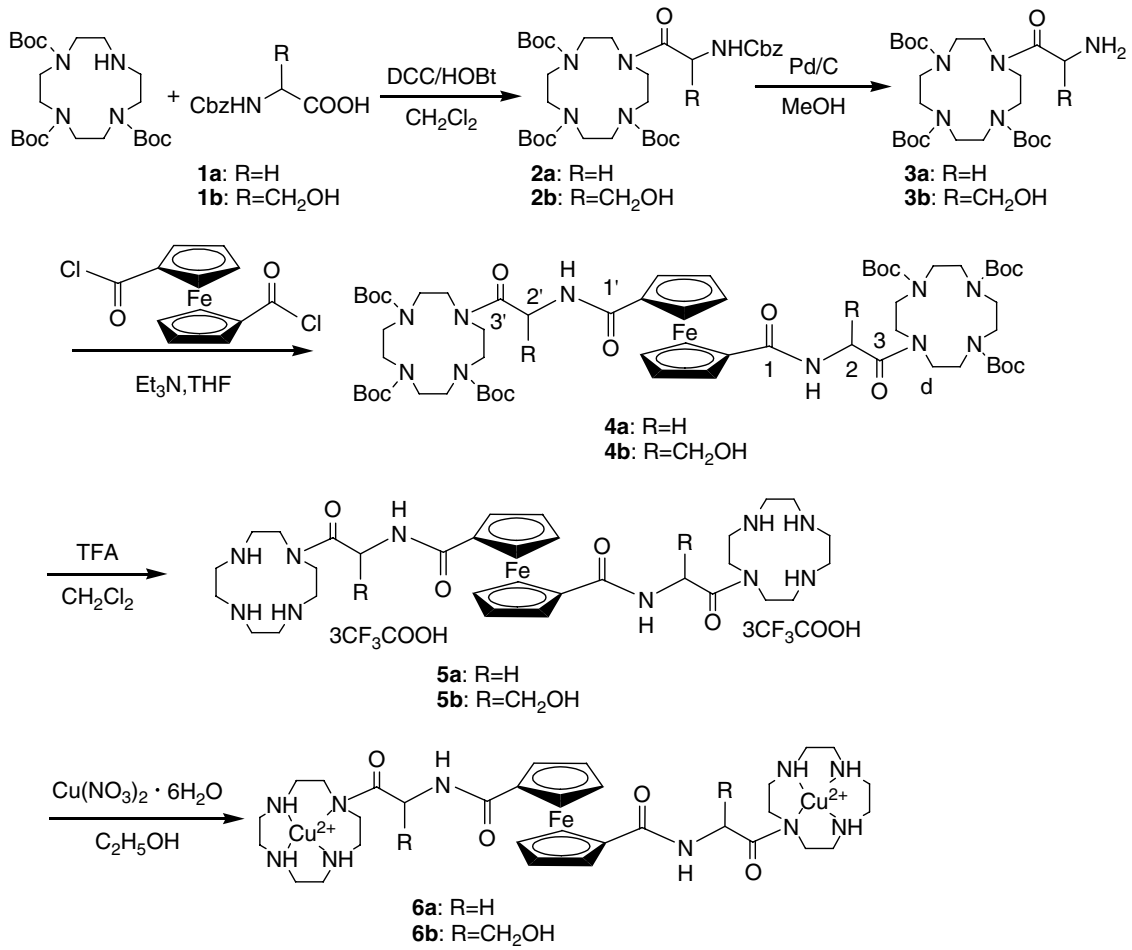
The synthetic route of the ferrocene-bridging dinuclear cyclen copper(II) complexes **6** was shown in Scheme 1. Firstly, *N*-Cbz-protected glycine or serine was introduced to 3Boc-cyclen to give compound **2**. Subsequent deprotection led to the formation of a terminal amine **3**, which could quickly and totally react with 1,1′-ferrocenedicarbonyl chloride to give the coupling product **4**. The protecting group (Boc) was then removed by trifluoroacetic acid (TFA), and the free ligand **5** was allowed to react with $Cu(NO_3)_2$ in ethanol to give the target complexes **6**. Here, we used amino acids to link the cyclen moiety to ferrocene. The reason was that peptides are ubiquitous in organisms and have good biocompatibility. Furthermore, the peptide bond can be easily modified. The complexes and intermediates were characterized by NMR, ESI-MS, IR and HRMS.

Catalytic cleavage of plasmid DNA

The cleavage of DNA catalyzed by different concentration of complexes **6a** and **6b** were studied. As shown in Fig. 1, the cleavage activity accelerated significantly associated with the increase in the concentration of complex. At a concentration of 112 μM, complex **6a** could almost totally convert the supercoiled DNA (form I) into nicked DNA (form II) and a small amount of linear DNA (form III). As to complex **6b**, a similar conversion of form I was observed with a lower concentration (36 μM). Therefore, for the catalytic cleavage activity, **6b** is superior to **6a**. This result might be due to the hydroxyl group, which could facilitate the production of reactive oxygen species responsible for DNA restriction.[21]

The time dependence of the DNA cleavage reaction catalyzed by complex **6** was then examined (Fig. 2). In order to observe the gradual change in the DNA cleavage course, the reaction time was lengthened to 40 min, and the concentrations of complex **6a** and **6b** were decreased to 30 and 20 μM, respectively. As shown in Fig. 2, the decrease in supercoiled DNA (form I) was accompanied by an increase in nicked DNA (form II). For the cleavage reactions catalyzed by **6b**, higher conversion could be achieved and a lower amount of complex was used than in the reactions catalyzed by **6a**.

The effect of radical scavengers on the DNA restriction was also examined. In order to identify the reactive oxygen species (ROS), which might be formed in the DNA cleavage process, experiments with a variety of radical scavengers were carried out. For example, sodium azide was used as s singlet oxygen scavenger, superoxide dismutase enzyme (SOD) was used as a superoxide scavenger, dimethyl sulfoxide (DMSO) and *tert*-butyl alcohol were used as scavengers of hydroxyl radicals. From the results shown in Fig. 3, DMSO, *tert*-butyl alcohol and SOD could partially inhibit the DNA cleavage process, while NaN_3 almost completely inhibited the cleavage. That is to say, singlet oxygen was the most reactive



Scheme 1. Synthetic route of target compounds.

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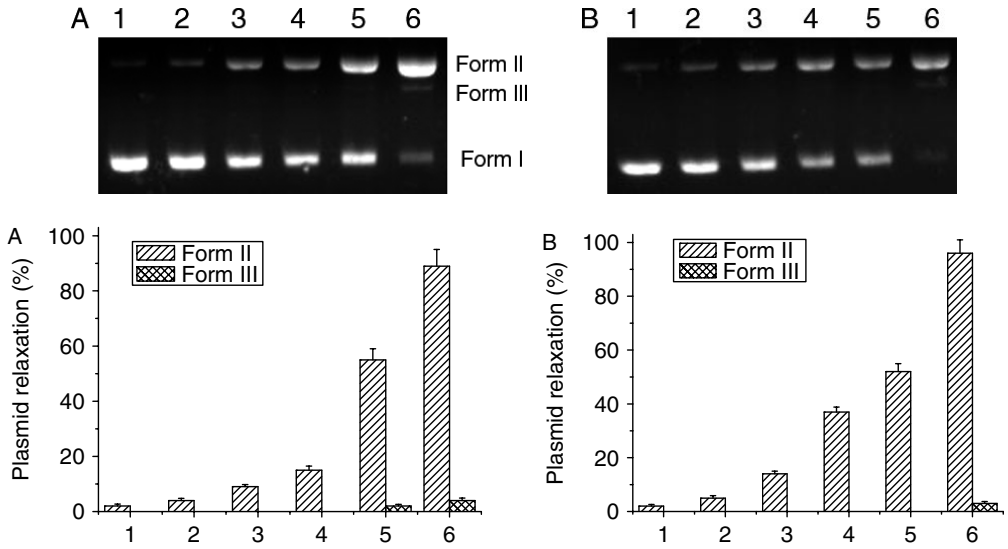

Figure 1. Agarose gel electrophoresis patterns for the cleavage pUC19 plasmid DNA (5 μg/ml) catalyzed by various concentrations of complexes **6a** (A) and **6b** (B), and their quantity of percentage plasmid relaxation (form II or form III) relative to plasmid DNA per lane. The reaction was carried out in an NaH_2PO_4–Na_2HPO_4 buffer (100 mM, pH 7.4) at 37 °C for 20 min. (A) Lane 1, DNA control; lanes 2–6: DNA + **6a** of 3.5, 7.0, 14.0, 28.0, 56.0 and 112.0 μM. (B) Lane 1: DNA alone; lanes 2–6: DNA + complex **6b** of 2.3, 4.5, 9.0, 18.0, 36.0 and 72.0 μM.

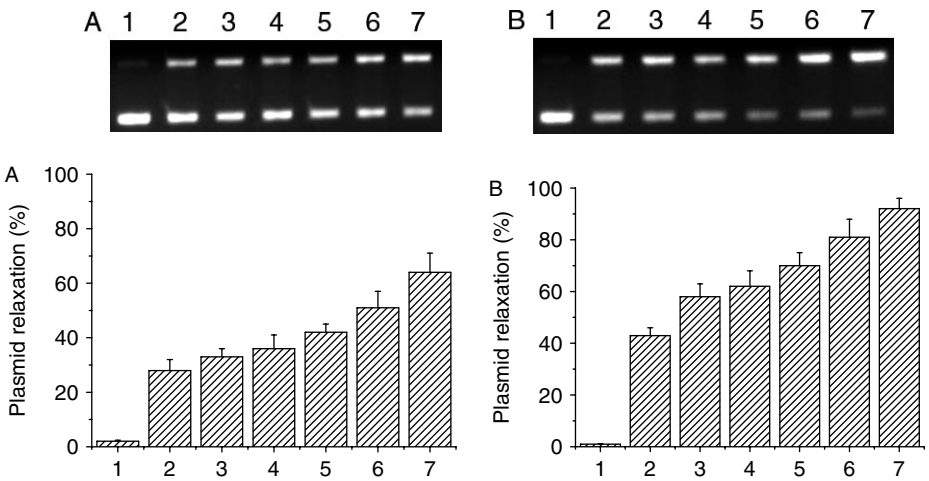

Figure 2. Effect of reaction time on the cleavage of pUC19 DNA (5 μg/ml) catalyzed by **6a** (30 μM) and **6b** (20 μM) in an Na_2HPO_4–NaH_2PO_4 buffer (100 mM, pH 7.4) at 37 °C. (A) Agarose gel electrophoresis diagrams of complex **6a**: lane 1, DNA control; lanes 2–7, DNA + complex **6a**, time = 3, 6, 10, 15, 20 and 40 min. (B) Agarose gel electrophoresis diagrams of complex **6b**: lane 1, DNA control; lanes 2–7, DNA + complex **6b**, time = 3, 6, 10, 15, 20 and 40 min.

oxygen species in the DNA cleavage reactions catalyzed by complex **6b**.[22]

Electrochemical studies of complex 6a

The oxidative DNA cleavage induced by metallonucleases often proceeds via redox cycles between different oxidation states of the transition metal ions. Therefore, the redox potential is a useful index for the evaluation of the cleavage ability.[23] As shown in Fig. 4, two irreversible electrochemical waves were found in the CV curve of complex **6a** in the absence of DNA. These waves corresponded to a Cu(I)–Cu(II) redox couple with a peak potential of 0.128 V (vs SCE) and 0.463 V, respectively. After the addition of DNA, one anodic peak potential shifted to 0.508 V, and the other shifted to 0.108 V, and the electric current also reduced at the same time. Such behaviors indicated the electrostatic interaction between complex **6a** and the saccharide–phosphate backbone of plasmid DNA.[23]

$$E_b^{o'} - E_f^{o'} = 0.0591 \log \frac{K_+}{K_{2+}}$$

Moreover, we used the equation to estimate the ratio of the binding constant of the Cu(I) and Cu(II) to DNA:

$$E_b^{o'} - E_f^{o'} = 0.0591 \times \log \frac{K_+}{K_{2+}}$$

where $E_b^{o'}$ and $E_f^{o'}$ are the formal potentials of the redox couple in the DNA bound and free form, respectively. For one copper atom in the complex **6a**, the anodic peak potential positively shifted 45 mV, so the ratio of K_+/K_{2+} could be calculated to be 5.77.

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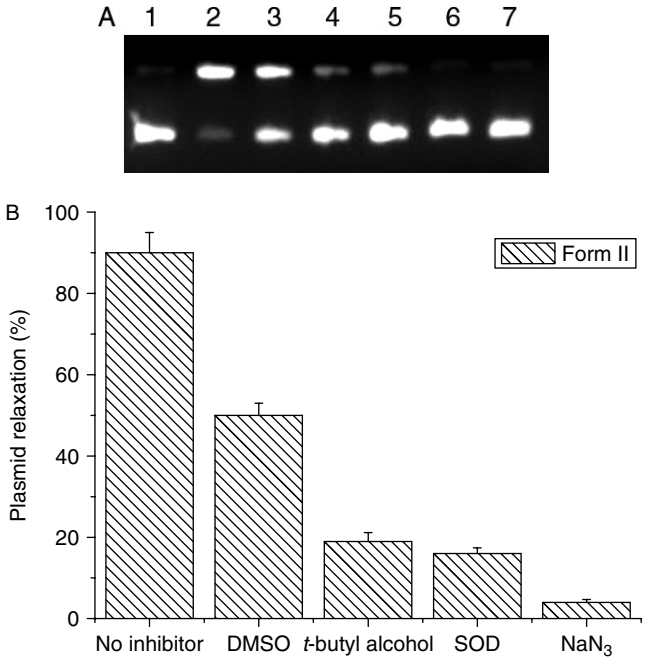

Figure 3. Inhibition studies on cleavage of pUC19 DNA (5 μg/ml) by complex **6b** (36 μM). reactions were carried out for 20 min as described above, except the inhibiter was added before the DNA to the system. Lane 1, DNA control; lane 2, DNA + **6b**; lane 3, DNA + **6b** + 143 mM of DMSO; lane 4: DNA + **6b** + 143 mM of *tert*-butyl alcohol; lane 5, DNA + **6b** + 143 mM of SOD; lane 6: DNA + **6b** + 143 mM of NaN_3; lane 7, DNA + cyclen Cu(II) complex. Here cyclen Cu(II) complex was used as control to the complex *6b*.

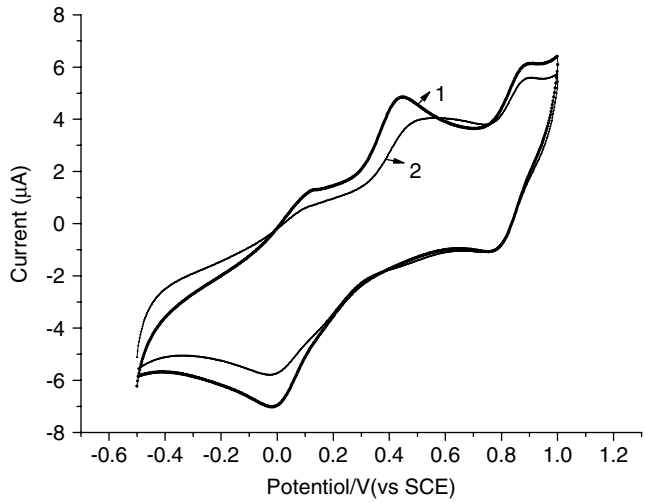

Figure 4. Cyclic voltammogram of **6a** in the absence (**1**) and presence (**2**) of CT DNA in KCl solution (pH = 7.4, 100 mM) at 100 mV/s.

For the other copper atom, the anodic peak potential negatively shifted 15 mV, and the ratio of K_{2+}/K_{+} was found to be 1.79. These results indicated that Cu(I) binding to DNA is more extensive than Cu(II) in the cleavage process, which wi in agreement with the reported results.[24]

Conclusions

Two novel dinuclear cyclen copper(II) complexes bridged by ferrocene were synthesized and their DNA cleavage ability was investigated. Complex **6b** exhibited better DNA damage activity than that of **6a**, and the DNA cleavage was catalyzed via an oxidative pathway without the use of any additives. Electrochemical studies showed the electrostatic interaction between complex **6a** and the saccharide-phosphate backbone of plasmid DNA. This kind of complex might serve as efficient 'self-activating' artificial nuclease and might have wide application in this area.

Experimental

General

All chemicals and reagents were obtained commercially and used as received. Anhydrous acetonitrile (CH_3CN), chloroforms ($CHCl_3$), dichloromethane (CH_2Cl_2) and tetrahydrofuran (THF) were dried and purified under nitrogen using standard methods and were distilled immediately before use. All aqueous solutions were

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prepared from deionized or distilled water. Electrophoresis-grade agarose and plasmid DNA (pUC19) were purchased from Takara Biotechnology Company, The disodium salt of calf thymus DNA (CT-DNA), and ethidium bromide (EB) were purchased from Sigma. The ^{1}H NMR spectra were measured on a Varian INOVA-400 spectrometer and the δ scale in ppm was referenced to residual solvent peaks or internal tetramethylsilane (TMS). HRMS-ESI spectra data were recorded on a Finnigan LCQDECA and a Bruker Daltonics Bio TOF mass spectrometer. IR spectra were recorded on a Shimadzu FTIR-4200 spectrometer as KBr pellets. Electrophoresis apparatus was a biomeans stack II-electrophoresis system, PPSV-010. Bands were visualized by UV light and photographed using a gel documentation system by the estimation of the intensity of the DNA bands, recorded on an Olympus Grab-IT 2.0 annotating image computer system. 1,1′-ferrocenedicarbonyl chloride and 3Boc-cyclen [1, 4, 7-tris(*tert*-butyl-oxycarbonyl)-1, 4, 7, 10-tetraazacyclododecance] were used as initial materials and synthesized according to the literature.[25]

Preparation of compound 2

In an ice bath, to a dry THF solution of **1** (**a**, 0.73 g/**b**, 0.83 g, 3.5 mmol), 3Boc-cyclen (1.42 g, 3.0 mmol) and 1-hydroxybenzotriazole (HOBt) (0.47 g, 3.5 mmol) was dropwise added *N*,*N*′-dicyclohexylcarbo-diimide (DCC, 0.72 g, 3.5 mmol) in 50 ml of THF. The resulting solution was stirred at 0 °C for 2 h and then warmed to room temperature and stirred overnight. The suspension was filtered and the precipitate was washed twice with a small amount of cold THF. The filtrate was evaporated *in vacuo* and the residue was purified by column chromatography (silica gel, EtOAC/hexane) to give **2** as a colorless amorphous solid.

2a: yield: 78%.^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ: 7.34–7.38 (m, 5H, Ph-H), 5.75 (s, 1H, N–H), 5.11 (s, 2H, $-CH_2-$Ph), 4.01 (d, J = 4.8 Hz, 2H, $-CH_2N-$), 3.42–3.46 (m, 16H, $-CH_2CH_2-$), 1.44–1.48 (m, 27H, Boc–H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 156.9 ($COOCH_2Ph$), 155.9 (*C*ONH), 155.5 (Boc, *C*O), 128.7, 128.5, 128.3, 127.5, 126.7 (Ph, *C*H), 80.1 [Boc, $C(CH_3)_3$], 50.8, 49.9, 49.6, 49.2 (cyclen, CH_2), 43.1 (CH_2NH), 28.5 (Boc, CH_3); HRMS-ESI: *m/z* calcd for $C_{33}H_{53}N_5NaO_9$ $[M + Na]^+$: 686.3735, found 686.3729.

2b: yield: 72%. ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ: 7.26–7.37 (m, 5H, Ph–H); 5.73 (d, J = 7.6 Hz, 1H, N–H); 5.12 (s, 2H, $-CH_2-$Ph); 4.69 (s, 1H, –OH); 3.92 (m, 1H, –CH); 3.81 (d, 2H, J = 8.2 Hz, $-CH_2OH$); 3.39–3.80 (m, 16H, $-CH_2CH_2-$); 1.44–1.48 (m, 27H, Boc–H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 157.1 ($COOCH_2Ph$), 155.7 (*C*ONH), 155.4 (Boc, *C*O), 128.6, 128.4, 128.3, 128.1, 126.8 (Ph, *C*H), 80.3, 79.4 [Boc, $C(CH_3)_3$], 64.9 (CH_2OH), 51.3 (CHNH), 50.4, 49.9, 49.4 (cyclen, CH_2), 28.4 (Boc, CH_3); HRMS-ESI: *m/z* calcd for $C_{34}H_{55}N_5NaO_{10}$ $[M+Na]^+$: 716.3841, found: 716.3868 $[M + Na]^+$.

Preparation of compound 3

Pd–C (0.10 g) was placed in a three-necked round bottom flask, and a solution of **2** (**a**, 1.00 g/**b**, 1.04 g, 1.5 mmol) in MeOH was injected into the flask under an N_2 atmosphere. The mixture was then stirred for 6 h under H_2 at room temperature. After filtration, the solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel, $CHCl_3$–MeOH) to give **3**.

3a, yield 80%. ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ: 3.37–3.46 (m, 18H, $-CH_2$, $-CH_2N$), 1.87 (s, 2H, NH_2), 1.46–1.48 (m, 27H, Boc–H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.0 ($CO-NH_2$), 157.1(Boc, *C*O), 80.2 [Boc, $C(CH_3)_3$], 49.2, 49.8 (cyclen,CH_2), 43.2 (CH_2NH_2), 28.4, 28.3 (Boc, CH_3); HRMS-ESI: *m/z* calcd for $C_{25}H_{48}N_5O_7$ $[M + H]^+$: 530.3548, found 530.3558.

3b: yield: 78%. ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ: 5.03 (s, 1H, –OH); 3.97 (t, J = 7.2 Hz, 1H, –CH); 3.79–3.83 (m, 2H, $-CH_2OH$); 3.37–3.71 (m, 16H,$-CH_2$); 1.45–1.46 (m, 27H, Boc–H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.5 (*C*ONH$_2$), 155.9 (Boc, *C*O), 80.4 [Boc, $C(CH_3)_3$], 65.6 (CH_2OH), 52.4 ($CHNH_2$), 49.8, 49.6 (cyclen,CH_2), 28.6, 28.5 (Boc, CH_3); HRMS-ESI: *m/z* calcd for $C_{26}H_{49}N_5O_8$: 560.3654 $[M + H]^+$, found 560.3650 $[M + H]^+$.

Preparation of compound 4

Under −20 °C in an ice–salt bath, to a dry THF solution of **3** (**a**, 1.06 g/**b**, 1.12 g, 2.0 mmol) and Et_3N (0.33 ml, 2.4 mmol) was dropwise added 1,1′-ferrocenedicarbonyl chloride (0.8 mmol) in 60 ml of THF. The resulting solution was stirred at −20 °C for 0.5 h and then warmed to room temperature for another 1 h. The suspension was filtered and the precipitate was washed twice with small amount of cold THF. The filtrate was evaporated *in vacuo* and the residue was purified by column chromatography (silica gel, EtOAC/hexane) to give **4** as yellow amorphous solid.

4a: yield: 78%. IR (KBr, cm^{-1}) υ: 3319, 2975, 2932, 1696, 1642, 1550, 1467, 1411, 1367, 1250, 1163, 778; ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ: 9.08 (s, 1H, NH), 8.94 (s, 1H, NH), 4.87 (s, 4H, ferrocene), 4.37 (t, 4H, J = 7.2 Hz, ferrocene), 4.08 (s, 4H, CH_2-), 3.96–3.48 (m, 32H, $-CH_2$), 1.48–1.52 (m, 54H, Boc–H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.6 (C_1, $C_{1'}$, *C*O), 169.3 (C_3,$C_{3'}$, *C*O), 162.4 (Boc, *C*O), 80.5 [Boc, $C(CH_3)_3$], 71.3, 70.4 (ferrocene, CH_2), 50.2, 50.0, 49.8, 49.4 (cyclen, CH_2), 42.8 (C_2, $C_{2'}$, CH_2), 29.5, 28.9, 28.5 (Boc, CH_3); HRMS-ESI: *m/z* calcd for $C_{62}H_{100}FeN_{10}NaO_{16}$ $[M+Na]^+$: 1319.6566; found 1319.6287.

4b: yield: 68%. IR (KBr, cm^{-1}) υ: 3430, 2975, 2929, 1696, 1633, 1540, 1466, 1411, 1366, 1249, 1165, 777.^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ: 5.19–5.18 (s, 2H,–OH); 4.87 (s, 2H, Ferrocene), 4.75 (s, 2H, ferrocene), 4.49 (s, 2 H, ferrocene), 4.34 (s, 2 H, ferrocene), 3.93 (s, 2 H, –CH); 3.88–3.38 (m, 36H, $-CH_2$,$-CH-$NH); 1.27–1.53 (m, 54H, Boc–H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.1 (C_1, $C_{1'}$, *C*O), 157.9, 157.1 (C_3,$C_{3'}$, *C*O), 155.7 (Boc, *C*O), 80.8, 80.4 [Boc, $C(CH_3)_3$], 71.7, 71.2, 69.9 (ferrocene, CH_2), 62.8 (CH_2OH), 52.8 (C_2, $C_{2'}$, *C*H), 50.0, 49.8, 49.3 (cyclen,CH_2), 29.6, 29.3, 28.5 28.4 (Boc, CH_3); HRMS-ESI: *m/z* calcd for $C_{64}H_{104}FeN_{10}NaO_{18}$ $[M + Na]^+$: 1379.6777, found 1379.6431.

Preparation of compound 5

To a stirred solution of **4** (**a**, 1.30 g/**b**, 1.36 g, 1 mmol) in CH_2Cl_2 (8 ml) was slowly added trifluoroacetic acid (6 ml) at room temperature, and the solution stirred for 2 h under N_2. Then, the reaction mixture was concentrated under reduced pressure below 40 °C to give crude product. The yellow oil was crystallized by anhydrous ether and washed three times with anhydrous ether (5 ml) to give yellow powder. The trifluoroacetic acid salts of ligand were used for the next step without further purification.

5a: yield: 88%. IR (KBr,cm^{-1}) υ: 3432, 2980, 2857, 1683, 1555, 1460, 1201, 1132, 832, 798, 721; ^{1}H NMR (400 MHz, D_2O) δ: 4.89 (s, 2H, ferrocene), 4.62 (s, 2H, ferrocene), 4.20 (s, 4H, ferrocene), 3.78–3.71 (m, 4H, $-CH_2$), 3.22–3.20 (m, 32H, CH_2N); ^{13}C NMR (100 MHz, D_2O) δ: 173.6 (C_1, $C_{1'}$, *C*O), 173.0 (C_3,$C_{3'}$, *C*O), 163.0 (Boc, *C*O), 74.3, 70.2, 65.9 (ferrocene, CH_2), 47.1 46.7, 46.2, 45.6, 44.9, 44.0, 43.7, 42.9, 42.7 (cyclen,CH_2), 41.8 (C_2, $C_{2'}$, CH_2); HRMS-ESI: *m/z* calcd for $C_{32}H_{52}FeN_{10}O_4$ $[M + H]^+$: 697.3601; found 697.3601.

5b: yield: 86%; IR (KBr, cm^{-1}) υ:3420, 3078, 2857, 1678, 1548, 1426, 1201, 1130, 834, 800, 721; ^{1}H NMR (400 MHz, D_2O) δ: 4.79

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(d, 4H, $J = 4.2$ Hz, ferrocene), 4.48 (s, 2H, ferrocene), 4.45 (s, 2H, ferrocene), 4.02 (d, 2H, $J = 8.0$ Hz, $-CH_2$), 3.83 (d, 2H, $J = 11.8$ Hz, $-CH_2$), 3.49 (d, 2H, $J = 7.8$ Hz, $-CH$), (m, 32H, $-CH-NH$); ^{13}C NMR (100 MHz, D_2O) δ: 174.5 (C_1, $C_{1'}$, *C*O), 173.6 (C_3,$C_{3'}$, *C*O), 163.4, 162.8 (Boc, *C*O), 73.8, 73.6, 70.0, 66.0 (ferrocene, CH_2), 60.3 (CH_2OH), 52.9 (C_2, $C_{2'}$, *C*H), 46.7, 46.2, 46.0, 45.6, 44.3, 42.9, 42.2 (cyclen,CH_2); HRMS-ESI: *m/z* calcd for $C_{34}H_{56}FeN_{10}O_6$ $[M+H]^+$: 757.3812; found 757.3812.

Preparation of complex 6

Excessive $Cu(NO_3)_2$ solid was added to the ethanol solution (20 ml) of **5**, and the mixture was stirred overnight under room temperature. The remaining residue was purified with centrifugal device, the yellow-green solids were washed with ethanol (3×6 ml).

6a: yield: 78%. IR (KBr, cm^{-1}) υ: 3415, 2932, 1638, 1382, 1037; anal. calcd for $C_{32}H_{52}Cu_2FeN_{12}O_{10} \bullet Cu(NO_3)_2$: C, 33.85; H, 4.62; N, 17.27. Found: C, 33.71; H, 5.10; N,17.16; MS-ESI: *m/z* calcd for $C_{32}H_{51}Cu_2FeN_{10}O_4$ $[M + 2NO_3 - H]^+$: 945.1793; found 945.0963.

6b: yield: 75%. IR (KBr, cm^{-1}) υ: 3404, 3240, 2974, 1632, 1542, 1381, 1039; anal. calcd for $C_{34}H_{56}Cu_2FeN_{10}O_6 \bullet 2Cu(NO_3)_2$: C, 32.44; H, 4.48; N, 15.58. Found: C, 32.58; H, 4.89; N, 15.47; MS-ESI: *m/z* calcd for $C_{34}H_{53}Cu_2FeKN_{11}O_5$ $[M - 3H + NO_3 + K]^+$: 980.2, found 980.1.

Electrochemistry

The redox potentials of **6a** were determined by cyclic voltammetry method using a conventional three-electrode system. A glass carbon electrode and a platinum wire were used as the working electrode and the counter-electrode, respectively. A saturated calomel electrode (SCE), which was separated from the test solution by the electrolytic solution sandwiched between two fritted disks and calibrated using the ferrocene–ferrocenium redox couple, was used as the reference electrode. KCl solution (0.1 M, pH 7.4) was used as the supporting electrolyte. The experiments were carried out in water at room temperature.

Cleavage of plasmid DNA

The DNA cleavage activity of complexes **6a** and **6b** was studied under physiological pH and temperature by gel electrophoresis (1% agarose) using supercoiled pUC 19 DNA as the substrate. In a typical experiment, pUC 19 DNA (5 µl, 0.018 µg/µl) in NaH_2PO_4–Na_2HPO_4 (100 mM, pH 7.4) was treated with different concentration of complexes **6a** and **6b**, followed by dilution with the NaH_2PO_4–Na_2HPO_4 buffer to a total volume of 17.5 µl. The samples were then incubated at 37 °C for different time, and loaded on a 1% agarose gel containing 1.0 µg/ml ethidium bromide. Electrophoresis was carried out at 40 V for 30 min in TAE buffer. Bands were visualized by UV light and photographed followed by the estimation of the intensity of the DNA bands using a gel documentation system.

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