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# Synthesis and DNA cleavage activities of mononuclear macrocyclic polyamine zinc(II), copper(II), cobalt(II) complexes which linked with uracil

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**Abstract**—Mononuclear macrocyclic polyamine zinc(II), copper(II), cobalt(II) complexes, which could attach to peptide nucleic acid (PNA), were synthesized as DNA cleavage agents. The structures of these new mononuclear complexes were identified by MS and $^1$H NMR spectroscopy. The catalytic activities on DNA cleavage of these mononuclear complexes with different central metals were subsequently studied, which showed that copper complex was better catalyst in the DNA cleavage process than zinc and cobalt complexes. The effects of reaction time, concentration of complexes were also investigated. The results indicated that the copper(II) complexes could catalyze the cleavage of supercoiled DNA (pUC 19 plasmid DNA) (Form I) under physiological conditions to produce selectively nicked DNA (Form II, no Form III produced) with high yields. The mechanism of the cleavage process was also studied.


## 1. Introduction

DNA is naturally stable polymer with an estimated half-life spontaneous hydrolysis of ~130,000 years under physiological conditions.[1] The main hindrance in DNA hydrolysis is the large negative charge that inhibits the attack of nucleophile to the DNA backbone. Studies on DNA hydrolysis agents are stimulated by the understanding of their potential application in biotechnology and as therapeutic agents.[2] Many groups reported different complexes,[3–13] which could accelerate the cleavage of DNA.[8] However, the catalytic efficiency of currently available chemical nucleases cannot be mentioned in the same breath as that of natural enzyme.

Recently, many polyamine macrocyclic ligands were studied because of their special properties. These ligands can produce both cationic and anionic complexes. The most widely studied ligand is 1,4,7,10-tetraazacyclododecane (cyclen), which has strong coordination ability toward a wide range of cations. Many cyclen complexes as chemical nucleases have been used in DNA recognition and cleavage.[14–22] So far the metal complex of cyclen is one kind of the most effective synthetic catalysts for DNA cleavage. We have synthesized several metal compounds containing cyclen unit and studied their catalytic abilities on DNA cleavage.[23–26] More recently, we reported the first synthesis of chiral peptide nucleic acid (PNA) monomer–cyclen conjugates.[27] There are many reports using the oligoPNA as recognition motif.[28,29] However, to the best of our knowledge, PNA monomer has not been reported in the literature. In these PNA monomer–cyclen conjugates, uridine could recognize adenine in the strand of DNA, and the cyclen moiety has high affinity with DNA. Hence we considered that these conjugates could be good catalysts in DNA cleavage. This prompted us to do more in-depth studies on the cleavage of DNA catalyzed by these kinds of complexes. Considering the π–π stacking effect will be helpful, we introduced phenyl group in the

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side chain.[30] In this paper, we describe the synthesis and characterization of noval PNA–cyclen metal complexes and their applications in the DNA cleavage. The result shows that the complexes can catalyze the DNA cleavage efficiently.

## 2. Results and discussion

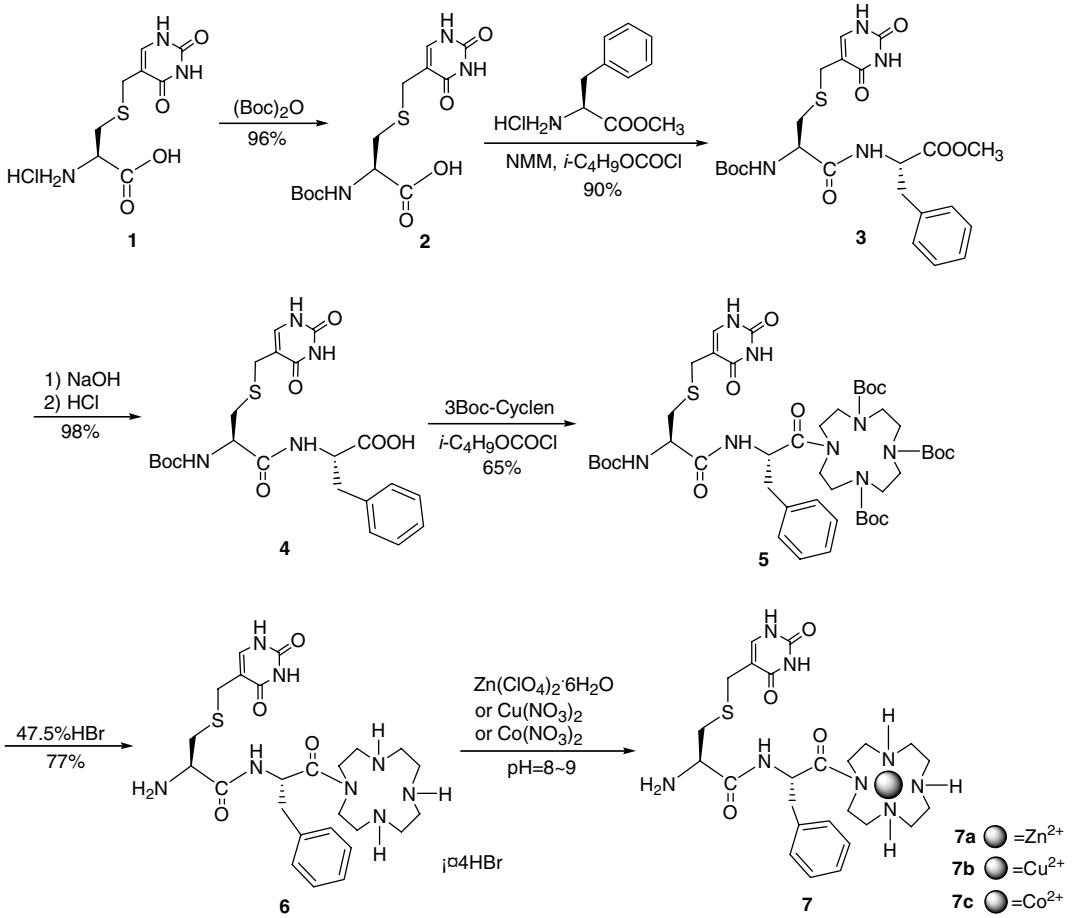
### 2.1. Preparation of the PNA–cyclen metal complexes

Scheme 1 shows the synthetic route of novel PNA–cyclen metal complexes **7**. *N*-*tert*-Butoxycarbonyl-thyminyl-cysteine **2** was prepared from 5-hydroxymethyluracil according to the literature procedure.[27] Compound **2** was used in a peptide coupling reaction with L-phenylalanine methyl ester, best results were achieved by using *N*-methyl morphine (NMM) and *i*-$C_4H_9OCOCl$ as acyl reagent. Deprotection of the methyl ester by NaOH and subsequent acidification gives **4**. Then the tri-Boc-protected cyclen was introduced by using *i*-$C_4H_9OCOCl$ as acyl reagent to form **5**. The hydrobromide salts of compound **6** were obtained by deprotection of **5** with 47.5% HBr in EtOH at low temperature. The salt was converted to the free based by adjusting pH to 8–9. The free ligand was allowed to react with $Zn(ClO_4)_2{\cdot}6H_2O$, $Cu(NO_3)_2$, or $Co(NO_3)_2$ in EtOH overnight to give the target Zn, Cu, Co complexes **7a**, **7b**, and **7c** in 68–76% yield as white, blue, and brown solid, respectively. These complexes were characterized by NMR, ESI-MS, and HRMS.

### 2.2. Cleavage of plasmid DNA

The cleavage activities of complexes **7a**–**c** with pUC 19 supercoiled DNA were studied. The cleavage of the supercoiled plasmid DNA (Form I) was processed under physiological conditions to produce open-circular form (Form II) selectively. The amounts of strand scission were assessed by agarose gel electrophoresis.

First, we compared the cleavage abilities of the PNA–cyclen metal complexes with different central metals. Figure 1 shows the results. It is obvious that Cu(II) complex (**7b**, lane 3) catalyzed the cleavage of plasmid DNA (pUC 19) much more efficiently than the Zn(II) (**7a**, lane 2) and Co(II) complex (**7c**, lane 4) under physiological conditions. Electrophoresis and densitometry indicated that single cleavage of the supercoiled form yielded 23%, 87%, and 31% nicked form, respectively, by **7a**–**c** (Fig. 1B). It is obvious that copper may provide additional and critical elements for the catalytic process. Although $Zn^{2+}$, $Cu^{2+}$, and $Co^{2+}$ have almost the same charge density (2.7, 2.8, and 2.8 $Å^{-1}$), $Cu^{2+}$ is a stronger Lewis acid based on their respective ionization




**Scheme 1.** Synthetic route of novel PNA–cyclen metal complexes **7**.

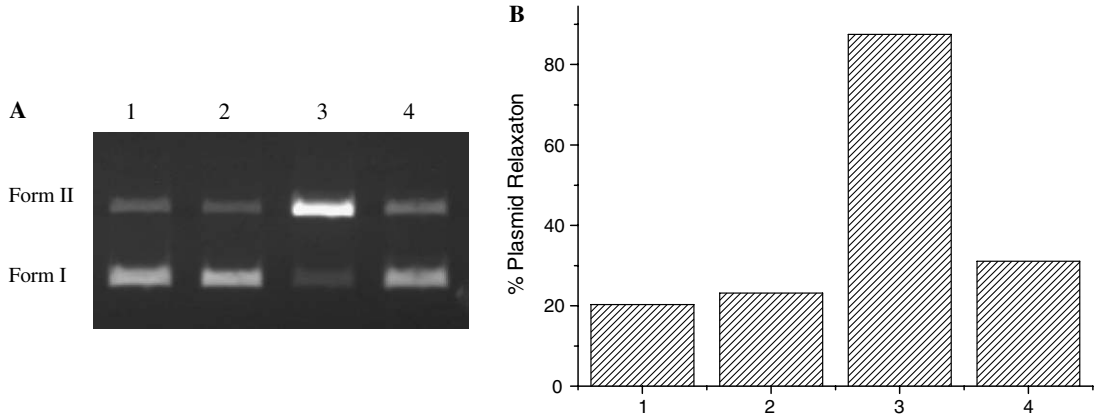



**Figure 1.** Effect of different complexes **7a**–**c** (0.143 mM) on the cleavage reactions of pUC 19 DNA (7 μg/mL) in a $NaH_2PO_4/Na_2HPO_4$ buffer (100 mM, pH 7.4) at 37 °C for 48 h. (A) Agarose gel electrophoresis diagram: lane 1, DNA control; lane 2, **7a**; lane 3, **7b**; lane 4, **7c**. (B) Quantitation of % plasmid relaxation relative to plasmid DNA per lane.

potentials ($I_{Cu^{2+}}$ = 20.29 eV, $I_{Zn^{2+}}$ = 17.96 eV, and $I_{Co^{2+}}$ = 17.06 eV).[31] The Lewis acidity of $Cu^{2+}$ might activate phosphodiester bond toward nucleophilic attack via charge neutralization, and, at the same time, might significantly lower the p$K_a$ of coordinated water molecule.[32–34] Our subsequent efforts focused on the reactivity of complex **7b** under physiological conditions.

A solution containing plasmid DNA was incubated in a 0.5 mL tube with catalyst **7b** at 37 °C, pH 7.0, as shown in Figure 2, the disappearance of supercoiled Form I was accompanied by appearance of open-circular Form II. An increase in the intensity of Form II was observed associated with the increase of reaction time. 69%, 90% nicked form (Form II) was observed when the reaction time is 12 and 48 h, respectively (Fig. 2B).

The cleavage of DNA by different concentrations of complex **7b** was also studied (Fig. 3). The amount of nicked DNA (Form II) observed in agarose gel electrophoresis diagram increased in accord with the change trend of the concentration of complex **7b** in the reaction

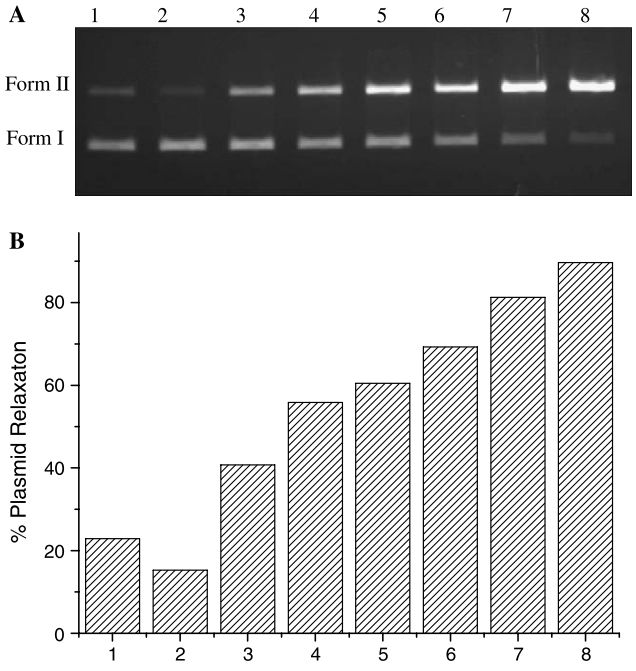



**Figure 2.** Effect of reaction time on the cleavage reaction of pUC 19 DNA (7 μg/mL) with complex **7b** (0.143 mM) in $NaH_2PO_4/Na_2HPO_4$ buffer (100 mM, pH 7.0) at 37 °C. (A) Agarose gel electrophoresis diagram: lane 1, DNA control, 48 h; lane 2, DNA control, 0 h; lane 3, 2 h; lane 4, 4 h; lane 5, 8 h; lane 6, 12 h; lane 7, 24 h; lane 8, 48 h. (B) Quantitation of % plasmid relaxation relative to plasmid DNA per lane.

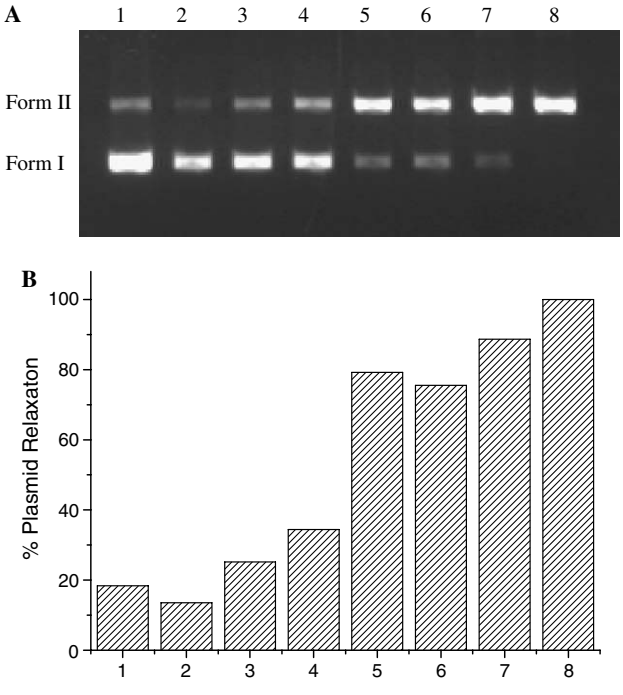



**Figure 3.** Effect of concentration of complex **7b** on the cleavage reactions of pUC 19 DNA (7 μg/mL) in a $NaH_2PO_4/Na_2HPO_4$ buffer (100 mM, pH 7.0) at 37 °C for 24 h. (A) Agarose gel electrophoresis diagram: lane 1, DNA control 24 h; lane 2, DNA control 0 h; lane 3, [**7b**] = 14.3 μM; lane 4, [**7b**] = 28.6 μM; lane 5, [**7b**] = 0.072 mM; lane 6, [**7b**] = 0.143 mM; lane 7, [**7b**] = 0.286 mM; lane 8, [**7b**] = 0.572 mM. (B) Quantitation of % plasmid relaxation relative to plasmid DNA per lane.

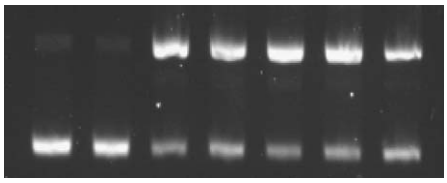



**Figure 4.** Effect of radical scavengers on the cleavage reaction of pUC 19 DNA (7 μg/mL) with complex **7b** (0.143 mM) in $NaH_2PO_4$/$Na_2HPO_4$ buffer (100 mM, pH 7.0) at 37 °C for 24 h. Agarose gel electrophoresis diagram: lane 1, DNA control, 24 h; lane 2, DNA control, 0 h; lane 3, **7b**; lane 4, **7b** + DMSO (1.43 mM); lane 5, **7b** + methol (1.43 mM); lane 6, **7b** + glycerol (1.43 mM); lane 7, **7b**+$NaN_3$ (1.43 mM).

system (Fig. 4A). Decreasing the concentration of **7b** in the order of 0.072 mM, 28.6 μM, and 14.3 μM results in 79%, 34%, and 25% nicked DNA, respectively. While increasing the concentration of **7b** to 0.572 mM, almost 100% nicked DNA was observed after 24 h (Fig. 3B).

The mechanism of the DNA cleavage catalyzed by **7b** was studied. Hydroxyl radical scavengers (1.43 mM DMSO, methol, glycerol) and oxygen scavenger (1.43 mM $NaN_3$) were introduced to the system. The free radical scavengers do not present any observable inhibition on the DNA cleavage reaction (Fig. 4). Therefore, DNA cleavage promoted by **7b** might occur through a hydrolytic pathway instead of an oxidative pathway.

The enzymatic religation of the nicked plasmid DNA with T4 DNA ligase was also studied, but we were unable to confirm any religation products. We tried to use TBARS-method[35] to detect hydroxyl radical, and no 532 nm absorbance was observed. It indicated that no hydroxyl radical emerged in this process.

In the Cu(II) complex **7b**, the uracil moiety in the PNA benefits the binding of the ligand to DNA, while the introduction of phenyl group in the side chain may open a way to stabilize the pretransition state. The role of copper ions in the cleavage of DNA may be multi-faceted, including the orientation of both the substrate and the water molecule, which acts as a nucleophile, and the activation of the water molecule via coordination. According to the experiment results, **7b** is an efficient catalyst, which could accelerate the DNA cleavage process under physiological conditions to produce selectively nicked DNA (Form II, no Form III produced) with high yields.

It has been reported that for mononuclear metal complexes, two water molecules coordinating to central metal are needed to achieve satisfying catalytic result of DNA cleavage.[36] However, copper(II) could provide only five-coordination structure, which means that in complex **7b**, the central metal can coordinate to only one water molecule. Therefore, it is interesting that mononuclear Cu(II) complex **7b** shows considerably high catalytic activity in the DNA cleavage process. More in-depth studies about the mechanism are underway in our laboratory.

## 3. Experimental

### 3.1. General

ESI-MS and HRMS spectral data were recorded on a Finnigan LCQDECA and a Bruker Daltonics Bio TOF mass spectrometer, respectively. The $^1$H NMR spectra were measured on a Varian INOVA-400 spectrometer and the $\delta$ scale in ppm was referenced to residual solvent peaks or internal tetramethylsilane (TMS). Polarimetric measurements were taken on a Perkin-Elmer-341 automatic polarimeter. UV–vis spectra were measured on a TU-1901 spectrophotometer. Melting points were determined using a micro-melting point apparatus and are uncorrected. DNA Gel Extraction Kit was purchased from V-gene Biotechnology limited. 5-Hydroxymethyluracil[37] and 1,4,7-tris(*tert*-butyloxycarbonyl)-1,4,7,10-tetraazacyclododecane (3Boc-cyclen)[38] were prepared according to the literature. All other chemicals and reagents were obtained commercially and were used without further purification.

### 3.2. Preparation of *S*-thyminyl-L-cysteine hydrochloride 1

A solution of L-cysteine hydrochloride (0.852 g, 6.00 mmol) and 5-hydroxymethyluracil (0.942 g, 6.00 mmol) in 2 N HCl (30 mL) was stirred at 50 °C for 48 h. After cooling, filtering, and evaporating the solvent under reduced pressure, the *S*-thyminyl-L-cysteine hydrochloride (1.59 g) was obtained as a white powder. Yield: 94%, mp 238–240 °C; $[\alpha]_D^{20}$ + 9.8 (*c* 1.0, $H_2O$); $^1$H NMR (400 MHz, $D_2O$, TMS) $\delta$: 7.64 (s, 1H, uracil-6-CH), 4.36–4.33 (m, 1H, $NH_2CH$), 3.59 (s, 2H, S–$CH_2$–uracil), 3.28–3.23 (m, 1H, $NCHCH_2S$), 3.15–3.09 (m, 1H, $NCHCH_2S$); ESI-MS: *m*/*z* = 244.0 [M−1−HCl]; HRMS (ESI) calcd for $C_8H_{11}N_3O_4SNa$ $[M+Na-HCl]^+$: *m*/*z* = 268.0362. Found: 268.0355.

### 3.3. Preparation of *N*-*tert*-butoxycarbonyl-*S*-thyminyl-L-cysteine (2)

To an aqueous solution of sodium hydrogen carbonate (1.68 g, 20.0 mmol) was added the solution of *S*-thyminyl-L-cysteine hydrochloride (1.12 g, 4.00 mmol) in 1,4-dioxane (60 mL). The solution was then cooled to 0 °C with an ice bath. Di-*tert*-butyl carbonate (2.62 g, 12.0 mmol) which was dissolved in 1,4-dioxane (20 mL) was added dropwise. After being kept at this temperature for another 20 h, the solution was allowed to warm to room temperature. The reaction mixture was then concentrated under reduced pressure and the pH was adjusted to 3–4 with 1 N $KHSO_4$. After cooling and filtering, compound **2** (1.324 g) was obtained as a white powder. Yield: 96%, mp 118–119 °C; $[\alpha]_D^{20}$ + 11.1 (*c* 1.0, $CH_3OH$); $^1$H NMR (400 MHz, DMSO) $\delta$: 12.82 (s, 1H, COOH), 11.14 (s,1H, uracil-1-NH), 10.85 (s, 1H, uracil-3-NH), 7.41 (s, 1H, uracil-6-CH), 6.92 (s, 1H, OCONH), 4.04–3.99 (m, 1H, Boc-

NHCH), 2.89–2.79 (m, 2H, S–$CH_2$–uracil), 2.73–2.65 (m, 2H, NCH$CH_2$S), 1.38 (s, 9H, C($CH_3$)$_3$); ESI-MS: $m/z$ = 344.4 $[M-1]^-$; HRMS (ESI) calcd for $C_{13}H_{19}N_3O_6SNa$ $[M+Na]^+$: $m/z$ = 368.0887. Found: 368.0889.

### 3.4. Preparation of compound 3

To a solution of *N*-*tert*-butyloxycarbonyl-*S*-thyminyl-L-cysteine **2** (1.03 g, 3.00 mmol) in anhydrous tetrahydrofuran (THF, 40 mL) was added *N*-methyl morphine (NMM) (0.390 mL, 3.30 mmol) and *i*-$C_4H_9OCOCl$ (0.402 mL, 3.00 mmol) sequentially at about −15 °C. After stirring for 10 min, a solution of L-phenylalanine methyl ester (3.30 mmol) and NMM (0.390 mL, 3.30 mmol) in anhydrous THF (60 mL) was poured into the reaction mixture. The mixture was stirred for another 0.5 h at −15 °C and left overnight at room temperature. After the solvent was evaporated under reduced pressure, the solid residue was dissolved in ethyl acetate and washed with saturated $NaHCO_3$, saturated brine and 2 N citric acid, the organic layer was dried with anhydrous $Na_2SO_4$. After the solvent was removed, the residue was purified by recrystallization from acetone and ethyl ether. Compound **3** (1.366 g) was obtained as a white powder. Yield: 90%, mp 210–212 °C; $^1$H NMR (400 MHz, DMSO) $\delta$: 11.17 (s, 1H, uracil-1-NH), 10.85 (d, 1H, $J$ = 4.0 Hz, uracil-3-NH), 8.29 (d, 1H, $J$ = 7.6 Hz, CONH), 7.40 (d, 1H, $J$ = 5.6 Hz, uracil-6-CH), 7.28–7.18 (m, 5H, Ph-H), 6.92 (d, 1H, $J$ = 8.4 Hz, OCONH), 4.49–4.44 (m, 1H, CHCOOCH$_3$), 4.15–4.09 (m, 1H, Boc-NHCH), 3.57 (s, 3H, OCH$_3$), 3.30–3.27 (m, 2H, Ph-$CH_2$), 3.05–2.91 (m, 2H, S–$CH_2$–uracil), 2.66–2.49 (m, 2H, NCH$CH_2$S), 1.37 (s, 9H, C($CH_3$)$_3$); ESI-MS: $m/z$ = 505.1 $[M-1]^-$.

### 3.5. Preparation of compound 4

2N Aqueous sodium hydroxide (5 mL, 10 mmol) was added dropwise to a suspension of compound **3** (2.00 mmol) in methanol (20 mL) at 0 °C. The mixture was stirred for 2 h at room temperature and the pH was adjusted to 7 with 1 N HCl. After removing most of the methanol, the pH was adjusted to about 2. The mixture was then extracted with ethyl acetate (3× 30 mL) and the organic layer was dried with anhydrous $Na_2SO_4$. After the solvent was evaporated under reduced pressure, compound **4** (0.964 g) was obtained as a white solid. Yield: 98%, mp 211–212 °C; $^1$H NMR (400 MHz, DMSO) $\delta$: 12.80 (s, 1H, COOH), 11.17 (s, 1H, uracil-1-NH), 10.84 (d, 1H, $J$ = 5.6 Hz, uracil-3-NH), 8.05 (d, 1H, $J$ = 7.6 Hz, CONH), 7.46 (d, 1H, $J$ = 8.4 Hz, uracil-6-CH), 7.39–7.19 (m, 5H, Ph-H), 6.94 (d, 1H, $J$ = 8.4 Hz, OCONH), 4.47–4.40 (m, 1H, CHCOOH), 4.16–4.12 (m, 1H, Boc-NHCH), 3.31–3.27 (m, 2H, Ph-$CH_2$), 2.56–2.54 (m, 2H, S–$CH_2$–uracil), 2.52–2.45 (m, 2H, NCH$CH_2$S), 1.37 (s, 9H, C($CH_3$)$_3$); ESI-MS: $m/z$ = 515.5 $[M+Na]^+$.

### 3.6. Preparation of compound 5

To a solution of compound **4** (1.00 mmol) in anhydrous THF (20 mL), *N*-methyl morphine (NMM) (0.130 mL, 1.10 mmol) and *i*-$C_4H_9OCOCl$ (0.134 mL, 1.00 mmol) were added at about −15 °C. After 10 min, a solution of 3Boc-cyclen (0.472 g, 1.00 mmol) in anhydrous THF (30 mL) was poured into the reaction mixture. The mixture was stirred for another 0.5 h at −15 °C and left overnight at room temperature. The mixture was then evaporated under reduced pressure to remove the solvent. The solid residue was dissolved in ethyl acetate and washed with saturated $NaHCO_3$, saturated brine, and 2 N citric acid, the organic layer was dried with anhydrous $Na_2SO_4$. After the solvent was evaporated, the residue was purified by column chromatography on silica gel (eluent: ethyl acetate/petroleum ether/acetone = 1:1:1, v/v/v) to afford compounds **5** (0.615 g) as a white solid. Yield: 65%, mp 141–143 °C; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 10.28 (s, 1H, uracil-1-NH), 9.88 (s, 1H, uracil-3-NH), 7.75 (s, 1H, CONH), 7.57 (s, 1H, uracil-6-CH), 7.37–7.24 (m, 5H, Ph-H), 5.59 (s, 1H, OCONH), 5.10–5.05 (m, 1H, CHCON), 4.54–4.50 (m, 1H, Boc-NHCH), 3.57–3.02 (m, 18H, Ph-$CH_2$, cyclen–$CH_2$), 2.81–2.79 (m, 2H, S–$CH_2$–uracil), 2.73–2.68 (m, 2H, NCH$CH_2$S), 1.50–1.41 (m, 36H, Boc-H); ESI-MS: $m/z$ = 970.1 $[M+Na]^+$.

### 3.7. Preparation of compound 6

To a solution of compound **5** (0.500 mmol) in ethanol (10 mL), 47.5% aqueous HBr (5 mL) was added dropwise. After being stirred at room temperature overnight, the reaction mixture was concentrated under reduced pressure to give the crude product, which was crystallized from ethanol/24% aqueous HBr to afford compound **6** (0.333 g) as a white powder. Yield: 77%, mp 239–241 °C; $^1$H NMR (400 MHz, $D_2O$) $\delta$: 7.63 (s, 1H, uracil-6-CH), 7.35–7.22 (m, 5H, Ph-H), 4.47–4.44 (m, 1H, CHCON), 4.30–4.24 (m, 1H, $NH_2$CH), 3.65–3.06 (m, 18H, Ph-$CH_2$, cyclen–$CH_2$), 3.01–2.95 (m, 2H, S–$CH_2$–uracil), 2.92–2.87 (m, 2H, NCH$CH_2$S); ESI-MS: $m/z$ = 547.6 $[M-4HBr+1]^+$.

### 3.8. Preparation of PNA–cyclen metal complexes (7)

To an aqueous solution (5 mL) of compound **6** (0.304 g, 0.350 mmol), an aqueous solution (5 mL) of $Zn(ClO_4)_2{\cdot}6H_2O$ (0.134 g, 0.360 mmol) was added slowly. The pH of the solution was adjusted to 8–9 with 1 N NaOH. After being stirred overnight, the reaction mixture was concentrated under reduced pressure. The obtained residue was crystallized from water to afford zinc(II) complex **7a** (0.215 g) as a white solid. Yield: 76%, mp 391–393 °C; $^1$H NMR (400 MHz, $D_2O$) $\delta$: 7.50–7.46 (m, 1H, uracil-6-CH), 7.13–7.05 (m, 5H, Ph-H), 4.18–4.15 (m, 1H, CHCON), 4.10–4.06 (m, 1H, $NH_2$CH), 3.49–2.88 (m, 18H, Ph-$CH_2$, cyclen–$CH_2$), 2.82–2.75 (m, 2H, S–$CH_2$–uracil), 2.70–2.66 (m, 2H, NCH$CH_2$S); ESI-MS: $m/z$ = 609.6 $[M-2ClO_4]^+$.

To an aqueous solution (5 mL) of compound **6** (0.304 g, 0.350 mmol), 1 N NaOH was added to adjust pH 8–9. $Cu(NO_3)_2$ (0.068 g, 0.360 mmol) was then added. The reaction mixture was stirred overnight and blue precipitate was formed. The reaction mixture was concentrated

under reduced pressure. The precipitate was centrifuged and washed by EtOH to afford **7b** (0.174 g) as blue solid. Yield: 68%, ESI-MS: $m/z$ = 608.2 $[M+Cu-H]^+$, HRMS (ESI) calcd for $C_{25}H_{37}N_8O_4SCu$ $[M+Cu-H]^+$: $m/z$ = 608.1949. Found: 608.1966.

To an aqueous solution (5 ml) of compound **6** (0.304 g, 0.350 mmol), 1 N NaOH was added to adjust pH 8–9. $Co(NO_3)_2$ (0.067 g, 0.360 mmol) was then added. The reaction mixture was stirred overnight and blue precipitate was formed. The reaction mixture was concentrated under reduced pressure. The precipitate was centrifuged and washed by EtOH to afford **7c** (0.181 g) as brown solid. Yield: 71%, ESI-MS: $m/z$ = 604.2$[M+Co-H]^+$.

### 3.9. Plasmid DNA cleavage

Plasmid DNA (pUC 19) cleavage activity of complexes **7a**–**c** was monitored by using agarose gel electrophoresis. In a typical experiment, supercoiled pUC 19 DNA (10 μL, 0.025 μg/μL) in $NaH_2PO_4/Na_2HPO_4$ (100 mM, pH 7.0) was treated with different concentrations of complexes **7a**–**c**, followed by dilution with the $NaH_2PO_4/Na_2HPO_4$ buffer to a total volume of 35 μL. The samples were then incubated at 37 °C for different times, 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.

### 3.10. Procedure for DNA ligation experiments

After incubation of pUC 19 DNA with 0.572 mM of **7b** for 24 h at 37 °C, the cleavage product was purified by DNA Gel Extraction Kit. The plasmid was then incubated for overnight at 16 °C with 10 U of T4 ligase in the buffer provided by the supplier.

### 3.11. TBARS-method

The reaction mixture, in a total volume of 2.4 mL, contained the following reagents at the final concentration stated: deoxyribose (0.8 mM); **7b** (0.14 mM); $Na_2HPO_4/NaH_2PO_4$ buffer (pH 7.0). The reaction mixture was incubated at 37 °C for 48 h. TBA (2 mL of 1% (w/v)) was then added, plus 2 mL of 2.8% (w/v) trichloroacetic acid. The whole was heated at 100 °C, cooled, and the absorbance of 532 nm was determined.

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## References and notes

1. Radzicka, A.; Wolfenden, R. *Science* **1995**, *267*, 90.
2. Pan, C. Q.; Lazarus, R. A. *Biochemistry* **1997**, *36*, 6624.
3. Hettich, R.; Schneider, H.-J. *J. Am. Chem. Soc.* **1997**, *119*, 5638.
4. Hettich, R.; Schneider, H.-J. *J. Chem. Soc. Perkin Trans 2.* **1997**, 2069.
5. Cowan, J. A. *Curr. Opin. Chem. Biol.* **2001**, *5*, 634.
6. Jin, Y.; Cowan, J. A. *J. Am. Chem. Soc.* **2005**, *127*, 8408.
7. Branum, M. E.; Tipton, A. K.; Zhu, S. R.; Que, L. *J. Am. Chem. Soc.* **2001**, *123*, 1898.
8. Jeung, C. S.; Kim, C. H.; Min, K.; Suh, S. W.; Suh, J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2401.
9. Dixon, N. E.; Geue, R. J.; Lamber, J. N.; Moghaddas, S.; Pearce, D. A.; Sargeson, A. M. *Chem. Commun.* **1996**, 1287.
10. Rammo, J.; Hettich, R.; Roigk, A.; Schneider, H. J. *Chem. Commun.* **1996**, 105.
11. Fitzsimons, M. P.; Barton, J. K. *J. Am. Chem. Soc.* **1997**, *119*, 3379.
12. Zhu, B.; Zhao, D.-Q.; Ni, J.-Z.; Zeng, Q.-H.; Huang, B.-Q.; Wang, Z.-L. *Inorg. Chem. Commun.* **1999**, *2*, 351.
13. Liu, C.-L.; Wang, M.; Zhang, T.-L.; Sun, H.-Z. *Coord. Chem. Rev.* **2004**, *248*, 147.
14. Aoki, S.; Kimura, E. *Chem. Rev.* **2004**, *104*, 769.
15. Kim, M.-S.; Suh, J. *Bull. Korean Chem. Soc.* **2005**, *26*, 1911.
16. Saki, N.; Akkaya, E. U. *J. Mol. Catal. A—Chem.* **2004**, *219*, 227.
17. Kimura, E.; Kitamura, H.; Ohtani, K.; Koike, T. *J. Am. Chem. Soc.* **2000**, *122*, 4668.
18. Kikuta, E.; Murata, M.; Katsube, N.; Koike, T.; Kimura, E. *J. Am. Chem. Soc.* **1999**, *121*, 5426.
19. Aoki, S.; Kagata, D.; Shiro, M.; Takeda, K.; Kimura, E. *J. Am. Chem. Soc.* **2004**, *126*, 13377.
20. Aoki, S.; Iwaida, K.; Hanamoto, N.; Shiro, M.; Kimura, E. *J. Am. Chem. Soc.* **2002**, *124*, 5256.
21. Aoki, S.; Kawatani, H.; Goto, T.; Kimura, E.; Shiro, M. *J. Am. Chem. Soc.* **2001**, *123*, 1123.
22. Khan, M. A.; Mustafa, J.; Musarrat, J. *Mutat. Res.* **2003**, *525*, 109.
23. Xiang, Q.-X.; Zhang, J.; Liu, P.-Y.; Xia, C.-Q.; Zhou, Z.-Y.; Xie, R.-G.; Yu, X.-Q. *J. Inorg. Biochem.* **2005**, *99*, 1661.
24. Peng, W.; Liu, P.-Y.; Jiang, N.; Lin, H.-H.; Zhang, G.-L.; Yu, X.-Q. *Bioorg. Chem.* **2005**, *33*, 374.
25. Chen, S.-Y.; Huang, Y.; Zhang, G.-L.; Cheng, H.; Xia, C.-Q.; Ma, L.-J.; Yu, H.; Yu, X.-Q. *Synthesis* **2005**, *6*, 888.
26. Li, Q.-L.; Huang, J.; Wang, Q.; Jiang, N.; Xia, C.-Q.; Lin, H.-H.; Wu, J.; Yu, X.-Q. *Bioorg. Med. Chem.* **2006**, *14*, 4151.
27. Ma, L.-J.; Zhang, G.-L.; Chen, S.-Y.; Wu, B.; You, J.-S.; Xia, C.-Q.; Yu, X-Q. *J. Peptide Sci.* **2005**, *11*, 812.
28. Zelder, F. H.; Mokhir, A. A.; Kraemer, R. *Inorg. Chem.* **2003**, *42*, 8618.
29. Bigey, P.; Sonnichsen, S. H.; Meunier, B.; Nielsen, P. E. *Bioconjugate Chem.* **1997**, *8*, 267.
30. Effects of the amino acid starting materials were studied before the work. The results showed that the complex with phenyl as side chain (derived from phenylalanine) behaved much better in the DNA cleavage than the complexes without aromatic side chain (serine and glycine were studied). When using the complexes derived from serine and glycine, very low conversions of substrate were observed.
31. *Handbook of Chemistry and Physics*, 78th ed; CRC Press: Boca Raton, FL, 1997.

32. Hegg, E. L.; Burstyn, J. N. *Coord. Chem. Rev.* **1998**, *173*, 133.
33. Lippard, S. J.; Berg, J. M. *Principles of Bioinorganic Chemistry*; University Science Books: Mill Valley, 1994, Chapter 2.
34. Sissi, C.; Mancin, F.; Gatos, M.; Palumbo, M.; Tecilla, P.; Tonellato, U. *Inorg. Chem.* **2005**, *44*, 2310.
35. Halliwell, B.; Gutteridge, J. M. C. *FEBS Lett.* **1981**, *128*, 347.
36. De Iuliis, G. N.; Lawrance, G. A.; Fieuw-Makaroff, S. *Inorg. Chem. Commun.* **2000**, *3*, 307.
37. Abdel-Rahman, A. A.-H.; El Ashry, E. S. H. *Synlett* **2002**, 2043.
38. Kimura, E.; Aoki, S.; Koike, T.; Shiro, M. *J. Am. Chem. Soc.* **1997**, *119*, 3068.