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Bioorganic Chemistry 33 (2005) 374–385
BIOORGANIC CHEMISTRY
www.elsevier.com/locate/bioorg

Dinuclear macrocyclic polyamine zinc(II) complexes linked with flexible spacers: Synthesis, characterization, and DNA cleavage

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Received 19 March 2005

Abstract

Dinuclear macrocyclic polyamine zinc(II) complexes, which have two cyclen groups linked by flexible spacers, have been synthesized as DNA cleavage agents. The structures of these new dinuclear complexes are consistent with the data obtained from elemental analysis, MS and ^{1}H NMR spectroscopy. The catalytic activity of these dinuclear complexes on DNA cleavage was studied. The results showed that the dinuclear zinc(II) complexes can catalyze the cleavage of supercoiled DNA (pUC 19 plasmid DNA) (Form I) under physiological conditions to produce selectively nicked DNA (Form II).

Keywords: 1,4,7,10-Tetraazacyclododecane (cyclen); Macrocyclic polyamine; Dinuclear complexes; pUC 19 DNA; Cleavage

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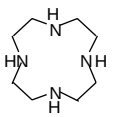
doi:10.1016/j.bioorg.2005.07.003

1. Introduction

There is great interest in the chemistry of macrocyclic polyamines due to their ability to interact with both metal cations and anionic species [1,2]. In particular, ligands based on 1,4,7,10-tetrazacyclododecane structure (**1**, cyclen), which contain different side arms have been the subject of intense study because of several successful applications of their metal complexes. For instance, these complexes are currently used in the separation of lanthanides [3], magnetic resonance imaging (MRI) contrast-enhancing agents [4], and radiopharmaceuticals [5]. In addition, such complexes could be used as models for the study and simulation of enzymes. Some zinc(II) complexes of azamacrocycles show dynamic anion recognition in water, mimicking the properties of some enzymes [6]. This has been used to achieve self assembly under physiological conditions [7] and even to control gene expression in vivo [8]. Schneider and our previous works show that macrocyclic polyamine metal complexes function as chemical nucleases and DNA cleaving agents [9].

It is also known that the metals in some many hydrolytic metalloenzymes exist as bi- and tri-nuclear complexes. The metal ions act in unison and cause rate accelerations as large as 10^{12}-fold [10]. This cooperative action of two or three metal centers has been mimicked in a number of model systems with varying degrees of success [11]. Scrimin and co-workers [12] demonstrated that the dinuclear zinc(II) complexes located on a peptide sequence can effectively catalyze the hydrolysis of plasmid DNA. Akkaya and co-workers synthesized a binuclear zinc(II) complex bridged by a 1,3-bis(methylene)benzene linker. Subsequent testing showed that the binuclear complex is highly efficient in the hydrolysis of plasmid DNA and, in comparison with a mononuclear complex, reveals a high degree of cooperativity between the two metal ion centers [13,14]. König et al. [15] reported urea derivatives of cyclen which were bridged bis-cyclens with aliphatic or aromatic spacers. However, the introduction of carbamoyl groups at the nitrogen atoms reduced their basicity and binding ability. They also studied the binding properties of these derivatives with double stranded DNA under physiological conditions.

Surprisingly, the dinuclear complexes of bis-cyclen as DNA cleavage agents linked with general flexible spacers, have not yet, to the best of our knowledge, been reported in the literature. In this paper, we report the synthesis, characterization, and properties of four dinuclear macrocyclic polyamine zinc(II) complexes linked with long flexible spacers. The synthetic method used for covalent bridging of cyclen is N-alkylation. It is well known that cleavage reactions can be increased by providing an additional nucleophilic group, such as a hydroxyl group [16]. Akkaya has shown that DNA/RNA hydrolysis can be accelerated by a zinc(II) ion containing a bound hydroxyl ion that acts as a nucleophilic center in the hydrolysis reaction [13]. Hence, we incorporated two β-hydroxyl groups into the spacers. The results shown that the dinuclear zinc(II) complexes more efficiently cleave plasmid DNA (pUC 19) under physiological conditions than the zinc(II) complex of the parent ligand **2**.



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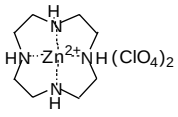

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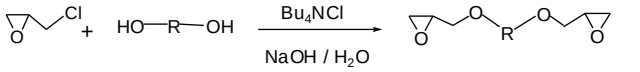
2. Results and discussion

2.1. Synthesis

Diglycidyl ethers **3**, the key intermediates in the synthesis of the four polyamine zinc(II) complexes, were obtained by Mouzin's one-step synthetic method in the presence of the phase transfer catalyst tetrabutylammonium chloride as shown in Scheme 1 [17,18]. The diglycidyl ethers **3** were purified by column chromatography to avoid rigorous Kugelrohr distillation under high vacuum. However, the yields of compounds **3a**–**d** were not high because undesired products such as monoglycidyl ethers and oligomers formed.

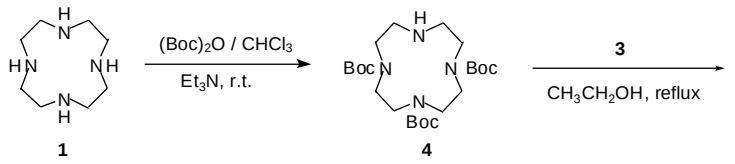
The Boc (*tert*-butyloxycarbonyl) group has been found to be an effective protecting group for the synthesis of some derivatives of cyclic polyamines [19]. Hence, 1,4,7-tris(*tert*-butyloxycarbonyl)cyclen (**4**, 3-Boc-cyclen) was used as the starting material. As outlined in Scheme 2, the reactions of **3a**–**d** with 2.15 equivalents of **4** in absolute alcohol slowly proceeded under refluxing conditions for 5 days to give 6-Boc-bis(cyclen) **5a**–**d**, which was purified by column chromatography to give purified compounds **5a**–**d** in 30–50% yield. To avoid the formation of the mono-cyclen, a slight excess of 3-Boc-cyclen **4** was added. TLC was used to monitor the reaction in order to determine when the reaction was complete.

The hydrobromide salts of compounds **6a**–**d** were obtained by deprotection of 6-Boc-bis(cyclen) **5a**–**d** with 47% aqueous HBr in EtOH at low temperature. The salts were converted to the free bases by passing them through an anion exchange column [20]. The free ligands **6a**–**d** were allowed to react with $Zn(ClO_4)_2{\cdot}6H_2O$ in EtOH. After cooling in the refrigerator for 12 h, the dinuclear macrocyclic polyamine zinc(II) complexes **7a**–**d** were obtained as colorless amorphous solid in 65–72% yield. The complexes were characterized by element analysis, ESI-MS, and NMR spectroscopy.

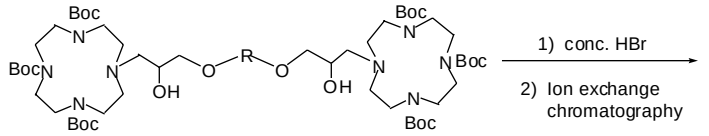


3 **a.** R=CH_2CH_2
b. R=$CH_2CH_2CH_2$
c. R=$CH_2CH_2CH_2CH_2$
d. R=$CH_2CH_2OCH_2CH_2$

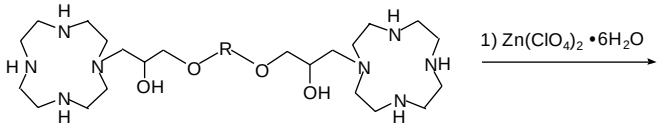
Scheme 1.



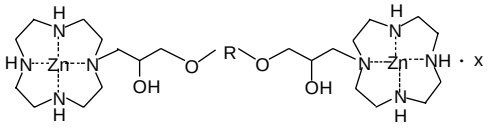
1 4



5 **a.** R=CH_2CH_2
b. R=$CH_2CH_2CH_2$
c. R=$CH_2CH_2CH_2CH_2$
d. R=$CH_2CH_2OCH_2CH_2$



6 **a.** R=CH_2CH_2
b. R=$CH_2CH_2CH_2$
c. R=$CH_2CH_2CH_2CH_2$
d. R=$CH_2CH_2OCH_2CH_2$



7 **a.** R=CH_2CH_2
b. R=$CH_2CH_2CH_2$
c. R=$CH_2CH_2CH_2CH_2$
d. R=$CH_2CH_2OCH_2CH_2$

x = $mClO_4 \cdot nOH$
m = 1,3 or 4 ; n = 0,1 or 3

Scheme 2.

2.2. Cleavage of plasmid DNA

The activities of complexes **7a**–**d** with pUC 19 supercoiled DNA were studied. The cleavage of the supercoiled plasmid DNA (Form I) was processed under physiological conditions to produce selectively open-circular form (Form II). The amounts of strand scission were assessed by agarose gel electrophoresis. Typical gels shown that the highest activity was observed at pH 7.8 and the results for these experiments are shown in Figs. 1–3.

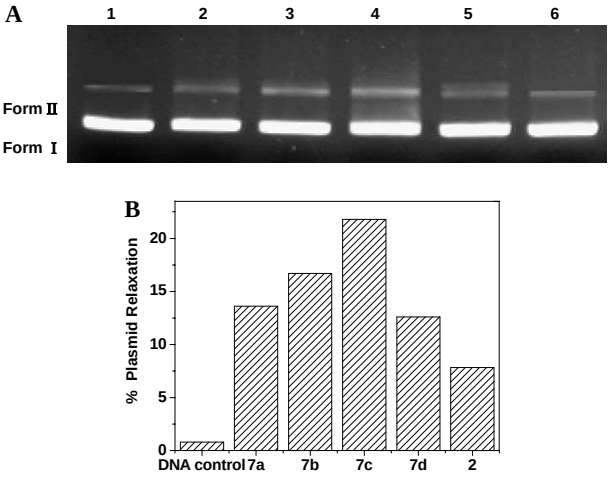


Fig. 1. Effect of different complexes **7a**–**d** and **2** (Zn^{2+}-cyclen) (1 mM) on the cleavage reactions of pUC 19 DNA (7 μg/mL) in a Tris–HCl buffer (100 mM, pH 7.8) at 37 °C for 12 h. (A) Agarose gel electrophoresis diagram: lane 1, DNA control; lane 2, **7a**; lane 3, **7b**; lane 4, **7c**; lane 5, **7d**; lane 6, **2**. (B) Quantitation of % plasmid relaxation relative to plasmid DNA per lane.

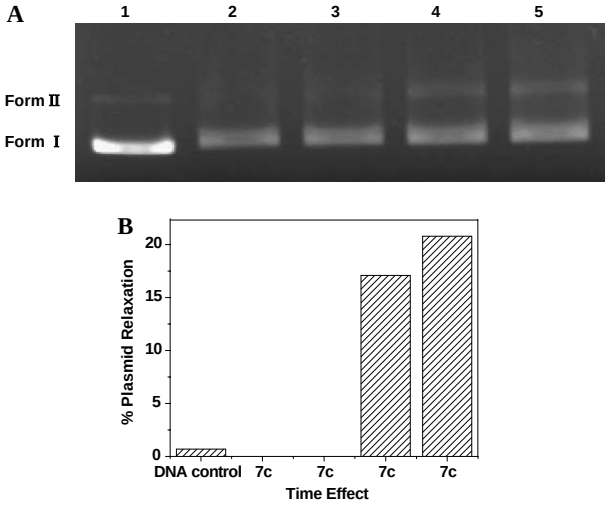


Fig. 2. Effect of time on the cleavage reaction of pUC 19 DNA (7 μg/mL) with complex **7c** (1 mM) in a Tris–HCl buffer (100 mM, pH 7.8) at 37 °C. (A) Agarose gel electrophoresis diagram: lane 1, DNA control, 10 h; lane 2, **7c**, 15 min; lane 3, **7c**, 1 h; lane 4,**7c**, 5 h; lane 5, **7c**, 10 h. (B) Quantitation of % plasmid relaxation relative to plasmid DNA per lane.

Figs. 1A and B show that dinuclear macrocyclic polyamine Zn(II) function as chemical nucleases and catalyze the cleavage of plasmid DNA (pUC 19) more efficiently than the zinc complex of the parent ligand **2**. To a certain extent, the cooperative activity of the two metal centers is established by comparing the cleavage of

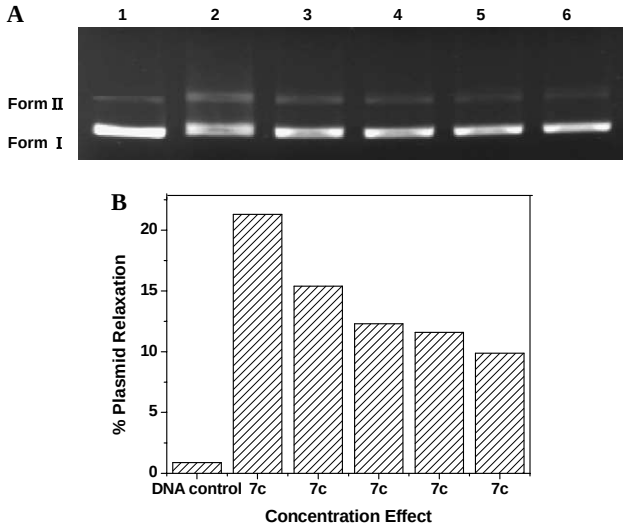


Fig. 3. Effect of concentration of complex **7c** on the cleavage reactions of pUC 19 DNA (7 μg/mL) in a Tris–HCl buffer (100 mM, pH 7.8) at 37 °C for 12 h. (A) Agarose gel electrophoresis diagram: lane 1, DNA control; lane 2, **7c**, 1 mM; lane 3, **7c**, 250 μM; lane 4, **7c**, 62.5 μM; lane 5, **7c**, 15.6 μM; lane 6, **7c**, 3.9 μM. (B) Quantitation of % plasmid relaxation relative to plasmid DNA per lane.

the plasmid DNA in the presence of **7a**–**d** to that of the zinc complex **2** (Zn^{2+}-cyclen), although this cooperative activity is weak. The results also showed that the mononuclear complex **2** at the same concentration did not result in any detectable hydrolysis, but efficient hydrolysis of plasmid DNA in the presence of **7a**–**d** was apparent. Electrophoresis and densitometry indicated that single cleavage of the supercoiled form yielded 13.6, 16.7, 21.8, and 12.6% nicked form, respectively, by **7a**–**d** (Fig. 1 B). In contrast, hydrolysis of supercoiled form in the presence of complex **2** could only give 7.8% nicked form. Compound **7c** is the best catalyst for DNA cleavage. The data suggest that the structures of the complexes, in particular the nature of bridging groups between the two cyclens, play an important role in the cleavage reaction. Our subsequent efforts focused on the reactivity of complex **7c** under physiological conditions.

A solution containing plasmid DNA was incubated in a 0.5 mL tube with catalyst **7c** at 37 °C, pH 7.8. As shown in Fig. 2, the disappearance of supercoiled Form I was accompanied by appearance of open circular Form II. An increase in the intensity of Form II resulted from an increase in the reaction time. When the reaction time reached 10 h, 20.8% nicked form (Form II) was observed (Fig. 2B).

The cleavage of DNA by different concentrations of complex **7c** was also studied (Fig. 3). The amount of nicked DNA (Form II) observed in agarose gel electrophoresis diagram decreased with the decrease of the concentration of complex **7c** in the reaction system (Fig. 3A). The concentration of **7c** decreased in the order of 1 mM, 250, 62.5, and 15.6 μM, resulting in 21.3, 15.4, 12.3, and 11.6% nicked DNA, respectively (Fig. 3B). When the concentration of **7c** fell to 3.9 μM (lane 6), there was some DNA cleavage (9.9% nicked DNA).

3. Experimental

3.1. General information

MS (ESI) mass spectral data were recorded on a Finnigan LCQ^{DECA} mass spectrometer. HRMS spectral data were recorded on Bruker Daltonics Bio TOF. ^{1}H NMR spectra were measured on a Varian INOVA-400 spectrometer and chemical shifts in ppm are reported relative to internal Me_4Si ($CDCl_3$) or (D_2O). ^{13}C NMR spectra were measured on a Bruker AC-E 200 spectrometer. Chemical shifts were reported in ppm relative to $CDCl_3$ ($\delta = 77.0$ ppm). Elemental analyses were performed using a Carlo-Elba 1106 elemental analytical instrument. Melting points were determined using a micro-melting point apparatus without any corrections. Electrophoresis apparatus was using a Biomeans Stack II-Electrophoresis system, PPSV-010. Bands were visualized by UV light and photographed followed by the estimation of the intensity of the DNA bands using a Gel Documentation System, recorded on an Olympus Grab-IT2.0 Annotating Image Computer System. All other chemicals and reagents were obtained commercially and used without further purification. Electrophoresis grade agarose and plasmid DNA (pUC 19) were purchased from Takara Biotechnology Company. Cyclen [21] **1**, Zn^{2+}-cyclen [8] **2** and 1,4,7-tris(*tert*-butyloxycarbonyl)-1,4,7,10-tetraazacyclododecance [22] **4** (3Boc-cyclen) were prepared as previously reported.

3.2. Synthesis of diglycidyl ether intermediates ***3a–d***

A mixture of epichlorhydrin (9.34 mL, 0.12 mol), sodium hydroxide pellets (4.8 g, 0.12 mol), water (0.5 mL, 0.028 mol), and tetrabutylammonium chloride (0.28 mL, 0.001 mol) was vigorously stirred. Glycol (1,2-ethandiol, 1,3-propanediol, 1,4-butanediol or diethylene glycol) (0.02 mol) cooled in ice was added dropwise. After the completion of addition, stirring was continued for another 45 min at 40 °C. The solid produced in the reaction process was filtered off and washed with dichloromethane. The combined organic layer was washed with saturated ammonium chloride to neutrality and dried with anhydrous magnesium sulfate. The solvent and excess epichlorhydrin were distilled off to give a yellow oil. The residue was purified by silica gel column chromatography to give **3a**–**d**.

3.2.1. Ethylene glycol diglycidyl ether (***3a***)

Colorless liquid; yield: 27.5%; eluent: ethyl acetate:petroleum ether (60–90 °C) = 5:1. MS (ESI): $m/z = 197.3$ $[M+Na]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 3.83–3.77 (m, 2H, OCH_2CH_2), 3.73–3.63 (m, 4H, OCH_2CH_2O), 3.45–3.39 (m, 2H, OCH_2CH_2), 3.20–3.10 [m, 2H, $2 \times CH_2(O)CH$], 2.81–2.61 [m, 4H, $2 \times CH(O)CH_2$].

3.2.2. 1,3-Propanediol diglycidyl ether (***3b***)

Colorless liquid; yield: 32.0%; eluent: chloroform: diethyl ether:methanol = 3:1:0.1. MS (ESI): $m/z = 189.1$ $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 3.76–3.70 (m, 2H, OCH_2CH_2), 3.65–3.54 (m, 4H, $OCH_2CH_2CH_2O$), 3.34–3.41 (m, 2H, OCH_2CH_2),

3.20–3.10 [m, 2H, 2 × CH_2(O)C*H*], 2.83–2.59 [m, 4H, 2 × CH(O)CH_2], 1.92–1.85 (m, 2H, OCH_2 CH_2 CH_2O).

*3.2.3. 1,4-Butanediol diglycidyl ether (**3c**)*

Colorless liquid; yield: 71.3%; eluent: ethyl acetate:petroleum ether (60–90 °C) = 3:1. MS (ESI): *m/z* = 203.2 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 3.75 (m, 8H, 4 × OCH_2), 3.17 [m, 2H, 2 × CH_2(O)C*H*], 2.62–2.42 [m, 4H, 2 × CH(O)CH_2], 1.50 (m, 4H, OCH_2 CH_2C$H_2$$CH_2O$).

*3.2.4. Diethylene glycol diglycidyl ether (**3d**)*

Colorless liquid; yield: 33.7%; eluent: ethyl acetate:petroleum ether (60–90 °C) = 6:1. MS (ESI): *m/z* = 253.3 $[M+Cl]^-$. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 3.82–3.77 (m, 2H, OC$H_2$$CH_2$), 3.72–3.63 (m, 8H, 2 × OCH_2CH_2O), 3.45–3.40 (m, 2H, OC$H_2$$CH_2$), 3.20–3.15 [m, 2H, 2 × CH_2(O)C*H*], 2.81–2.60 [m, 4H, 2 × CH(O)CH_2].

*3.3. General procedure for the synthesis of **5a–d***

Under a N_2 atmosphere, a solution of diglycidyl ether **3** (1.48 mmol) and 1,4,7-tris(*tert*-butyloxycarbonyl)-1,4,7,10-tetraazacyclododecane **4** (1.5 g, 3.17 mmol) in 60 mL of absolute alcohol was stirred at refluxing condition. The progress of the reaction was monitored by TLC. After 5 days, the reaction was almost complete. After evaporating the solvent under reduced pressure, the residue was passed through a silica gel chromatography to afford **5a–d**.

*3.3.1. Ethylene glycol-O,O′-[2,2′-dihydroxy-3,3′- bis(4,7,10-tris(tert-butyloxycarbonyl)-1,4,7,10-tetraazacyclododecane)] dipropyl ether (**5a**)*

White solid; yield: 53.2%; m.p. 56–58 °C; eluent: ethyl acetate:petroleum ether (60–90 °C):methanol = 3:1:0.1. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 3.95–3.90 [m, 2H, 2 × NCH_2C*H*(OH)], 3.65–3.59 (m, 8H, 4 × OCH_2), 3.53–3.35 (m, 24H, 6 × NCH_2CH_2N), 3.19 (br s, 2H, 2 × O*H*), 2.80–2.30 [m, 12H, 2 × CH_2N(CH_2)CH_2], 1.45 [s, 54H, 6 × OC(CH_3)$_3$]. ^{13}C NMR (200 MHz, $CDCl_3$): δ (ppm) = 156.3, 155.5 (C═O), 79.7, 79.4 [*C*(CH_3)$_3$], 74.4, 72.6, 71.5, 70.6, 69.4 (O*C*H_2), 67.5, 66.8 [*C*(OH)], 57.5, 55.7, 49.9, 48.1 (N*C*H_2), 28.6, 28.4 [C(*C*H_3)$_3$]. MS (ESI): *m/z* = 1119.54 $[M + H]^+$. HRMS: found *m/z* = 1119.7487 $[M + H]^+$, $C_{54}H_{103}N_8O_{16}$ requires 1119.7487.

*3.3.2. 1,3-Propanediol-O,O′-[2,2′-dihydroxy-3,3′-bis(4,7,10-tris(tert-butyloxycarbonyl) -1,4,7,10-tetraazacyclododecane)] dipropyl ether (**5b**)*

White solid; yield: 32.2%; m.p.: 54–56 °C; eluent: ethyl acetate:petroleum ether (60–90 °C):methanol = 3:1:0.1. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 3.98–3.88 [m, 2H, 2 × NCH_2C*H*(OH)], 3.66–3.30 (m, 8H, 4 × OCH_2; m, 24H, 6 × NCH_2CH_2N), 3.15 (br s, 2H, 2 × O*H*), 2.93–2.30 [m, 12H, 2 × CH_2N(CH_2)CH_2], 1.90–1.80 [m, 2H, CH_2(CH_2)$_2$], 1.45 [s, 54H, 6 × OC(CH_3)$_3$]. ^{13}C NMR (200 MHz, $CDCl_3$): δ (ppm) = 155.4 (C═O), 79.6, 79.2 [*C*(CH_3)$_3$], 74.0, 72.0, 71.5, (O*C*H_2), 69.3, 68.3, 67.6, 66.7 [*C*(OH)], 57.6, 56.6, 55.7, 49.9, 48.1 (N*C*H_2), 29.6 (OCH_2*C*$H_2$$CH_2O$), 28.5, 28.4

[C(CH_3)$_3$]. MS (ESI): $m/z = 1133.4$ $[M + H]^+$. HRMS: found $m/z = 1155.7451$ $[M + Na]^+$, $C_{55}H_{104}N_8Na_1O_{16}$ requires 1155.7463.

*3.3.3. 1,4-Butanediol-O,O′- [2,2′-dihydroxy-3,3′-bis(4,7,10-tris(tert-butyloxycarbonyl)-1,4,7,10-tetraazacyclododecane)] dipropyl ether (**5c**)*

White solid; yield: 32.3%; m.p.: 59–61 °C; eluent: dichloromethane: acetone:methanol = 4:3:0.1. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 3.99–3.88 [m, 2H, 2 × NCH$_2$C*H*(OH)], 3.60–3.30 (m, 8H, 4 × OCH_2; m, 24H, 6 × NCH_2CH_2N), 3.12 (br s, 2H, 2 × O*H*), 2.96–2.34 [m, 12H, 2 × CH_2N(CH_2)CH_2], 1.63–1.58 (m, 4H, CH$_2$CH_2CH_2CH$_2$), 1.45 [s, 54H, 6 × OC(CH_3)$_3$]. ^{13}C NMR (200 MHz, $CDCl_3$): δ (ppm) = 156.2, 155.4 (C═O), 79.6, 79.3 [*C*(CH$_3$)$_3$], 73.9, 71.9, 71.6, 71.2 (O*C*H$_2$), 67.7, 66.8 [*C*(OH)], 57.8, 56.6, 55.8, 49.9, 48.1 (N*C*H$_2$), 28.6, 28.4 [C(*C*H$_3$)$_3$], 26.3 (CH$_2$*C*H$_2$*C*H$_2$CH$_2$). MS (ESI): $m/z = 1147.6$ $[M + H]^+$. HRMS: found $m/z = 1147.7738$ $[M + H]^+$, $C_{56}H_{107}N_8O_{16}$ requires 1147.7800.

*3.3.4. Diethylene glycol-O,O′- [2,2′-dihydroxy-3,3′- bis(4,7,10-tris(tert-butyloxycarbonyl)-1,4,7,10-tetraazacyclododecane)]dipropyl ether (**5d**)*

White solid; yield: 31.8%; m.p.: 52–54 °C; eluent: ethyl acetate:petroleum ether (60–90 °C):methanol = 3:1:0.1. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 4.05–3.91 [m, 2H, 2 × NCH$_2$C*H*(OH)], 3.71–3.59 (m, 12H, 6 × OCH_2), 3.58–3.32 (m, 24H, 6 × NCH_2CH_2N), 3.24 (br s, 2H, 2 × O*H*), 2.93–2.38 [m, 12H, 2 × CH_2N(CH_2)CH_2], 1.45 [s, 54H, 6 × OC(CH_3)$_3$]. ^{13}C NMR (200 MHz, $CDCl_3$): δ (ppm) = 155.4 (C═O), 79.5, 79.2 [*C*(CH$_3$)$_3$], 74.8, 74.6, 73.1, 71.3, 70.4, 69.3 (O*C*H$_2$), 67.4, 66.7 [*C*(OH)], 56.9, 49.8, 48.0 (N*C*H$_2$), 28.6, 28.4 [C(*C*H$_3$)$_3$]. MS (ESI): $m/z = 1163.5$ $[M + H]^+$. HRMS: found $m/z = 1185.7612$ $[M + Na]^+$, $C_{56}H_{106}N_8Na_1O_{17}$ requires 1185.7568.

*3.4. General procedure for the synthesis of **6a–d***

To a solution of 6-Boc-bis(cyclen) **5** (0.25 mmol) and ethanol (6 mL), 47% aqueous HBr (3 mL) was added slowly at 0 °C. After the reaction mixture stirred overnight at room temperature, the solvents were evaporated in vacuo below 40 °C and the residue was recrystallized from H_2O/EtOH to afford the hexahydrobromide salt of **6** as a white solid. Compounds **6a–d**·6HBr was passed through an anion exchange resin column with water to obtain free ligands **6a–d**.

*3.4.1. Ethylene glycol-O,O′- [2,2′-dihydroxy-3,3′-bis(1,4,7,10-tetraazacyclododecane)] dipropyl ether (**6a**)*

Colorless amorphous solid; yield: 73.5%. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 3.94–3.85 [m, 2H, 2 × NCH$_2$C*H*(OH)], 3.74–3.50 (m, 8H, 4 × OCH_2), 3.10 (br s, 2H, 2 × O*H*), 2.96–2.38 [m, 6H, 6 × N*H*(CH$_2$)$_2$; m, 32H, 4 × N(CH_2CH_2)$_2$; d, 4H, 2 × NCH_2CH(OH)]. ^{13}C NMR (200 MHz, $CDCl_3$): δ (ppm) = 74.2, 71.1, 70.8, 70.5 (O*C*H$_2$), 68.2, 68.1 [*C*(OH)], 57.6, 57.3, 57.2, 52.3, 52.0, 48.5, 47.8, 46.7, 46.2, 45.0, 44.8, 44.1 (N*C*H$_2$). MS (ESI): $m/z = 541.6$ $[M + Na]^+$. HRMS: found $m/z = 519.4335$ $[M + H]^+$, $C_{24}H_{55}N_8O_4$ requires 519.4341.

*3.4.2. 1,3-Propanediol-O,O′- [2,2-dihydroxy-3,3-bis(1,4,7,10-tetraazacyclododecane)] dipropyl ether (**6b**)*

Colorless amorphous solid; yield: 84.6%. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 3.96–3.84 [m, 2H, 2 × $NCH_2CH(OH)$], 3.65–3.41 [m, 8H, 4 × OCH_2; m, 6H, 6 × $NH(CH_2)_2$], 3.07 (br s, 2H, 2 × OH), 2.84–2.42 [m, 32H, 4 × $N(CH_2CH_2)_2$; d, 4H, 2 × $NCH_2CH(OH)$], 1.88–1.75 [m, 2H, $CH_2(CH_2)_2$]. ^{13}C NMR (200 MHz, $CDCl_3$): δ (ppm) = 76.4, 73.7, 73.5, 73.2, 72.9, 72.4, 72.0 (OCH_2), 68.4, 68.2, 68.0, 67.9 [*C*(OH)], 57.8, 57.5, 52.3, 49.7, 46.7, 46.6, 46.5, 46.1, 45.9, 45.0, 44.8 (NCH_2), 29.5 ($CH_2CH_2CH_2$). MS (ESI): *m*/*z* = 533.5 $[M+H]^+$. HRMS: found *m*/*z* = 533.4389 $[M+H]^+$, $C_{25}H_{57}N_8O_4$ requires 533.4503.

*3.4.3. 1,4-Butanediol-O,O′-[2,2′-dihydroxy-3,3′-bis(1,4,7,10-tetraazacyclododecane)]dipropyl ether (**6c**)*

Colorless amorphous solid; yield: 76.3%. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 3.92–3.86 [m, 2H, 2 × $NCH_2CH(OH)$], 3.53–3.38 (m, 8H, 4 × OCH_2), 3.29–2.84 [br s, 2H, 2 × OH; m, 6H, 6 × $NH(CH_2)_2$], 2.83–2.43 [m, 32H, 4 × $N(CH_2CH_2)_2$; d, 4H, 2 × $NCH_2CH(OH)$], 1.68–1.62 (m, 4H, $CH_2CH_2CH_2CH_2$). ^{13}C NMR (200 MHz, $CDCl_3$): δ (ppm) = 73.6, 73.3, 72.2, 72.0, 71.2, 71.1 (OCH_2), 69.2, 68.4, 68.2, 68.0 [*C*(OH)], 58.3, 57.9, 57.7, 52.6, 47.9, 47.0, 46.8, 46.4, 46.2, 45.4 (NCH_2), 26.3 ($CH_2CH_2CH_2CH_2$). MS (ESI): *m*/*z* = 547.6 $[M+H]^+$.

*3.4.4. Diethylene glycol-O,O′-[2,2′-dihydroxy-3,3′-bis(1,4,7,10-tetraazacyclododecane)] dipropyl ether (**6d**)*

Colorless amorphous solid; yield: 94.0%. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 3.96–3.86 [m, 2H, 2 × $NCH_2CH(OH)$], 3.70–3.39 [m, 12H, 6 × OCH_2; m, 6H, 6 × $NH(CH_2)_2$], 3.20–3.04 (br s, 2 H, 2 × OH), 2.88–2.41 [m, 32H, 4 × $N(CH_2CH_2)_2$; d, 4H, 2 × $NCH_2CH(OH)$]. ^{13}C NMR (200 MHz, $CDCl_3$): δ (ppm) = 76.3, 74.3, 74.0, 73.9, 73.3, 73.2, 71.8, 70.4 (OCH_2), 68.9, 68.4, 68.1, 66.7 [*C*(OH)], 57.6, 57.5, 57.3, 57.2, 52.3, 48.5, 47.7, 46.7, 46.1, 45.0, 44.9 (NCH_2). MS (ESI): *m*/*z* = 563.6 $[M+H]^+$.

*3.5. General procedure for the synthesis of 7**a**–**d***

To a 10 mL EtOH solution of free ligand **6** (0.20 mmol) obtained as described above, $Zn(ClO_4)_2 \cdot 6H_2O$ (0.42 mmol) was added. The resulting solution stirred at room temperate for 1 h. After being cooled, the solid was filtered off, washed with absolute alcohol to give the dinuclear zinc(II) complexes **7a**–**d**.

*3.5.1. Ethylene glycol-O,O′-[2,2′-dihydroxy-3,3′-bis(1,4,7,10-tetraazacyclododecane)]dipropyl ether dizinc(**II**) complex (**7a**)*

Colorless amorphous solid; yield: 68.4%. ^{1}H NMR (400 MHz, D_2O): δ (ppm) = 4.18–4.09 [m, 2H, 2 × $NCH_2CH(OH)$], 3.77–3.48 (m, 8H, 4 × OCH_2), 3.24–2.68 (m, 32H, 8 × NCH_2CH_2N; m, 4H, 2 × NCH_2CH). MS (ESI): *m*/*z* = 745.3 $[M-3ClO_4-H_2O]^+$. Anal Calcd. for $C_{24}H_{54}N_8O_4Zn_2 \cdot 4ClO_4 \cdot H_2O$ (1065.33): C, 27.03; H, 5.26; N, 10.51, Found: C, 27.33; H, 5.61; N, 10.31. Atomic absorption spectrometry for Zn%: 12.27, Found: 12.17.

*3.5.2. 1,3-Propanediol-O,O′-[2,2′-dihydroxy-3,3′-bis(1,4,7,10-tetraazacyclododecane)] dipropyl ether dizinc(**II**) complex (**7b**)*

Colorless amorphous solid; yield: 72.0%. ^{1}H NMR (400 MHz, D_2O): δ (ppm) = 4.20–4.12 [m, 2 H, 2 × NCH_2CH(OH)], 3.68–3.40 (m, 8 H, 4 × OCH_2), 3.23–2.68 (m, 32 H, 8 × NCH_2CH_2N; m, 4H, 2 × NCH_2CH), 1.92–1.82 (m, 2H, $CH_2CH_2CH_2$). MS (ESI): m/z = 861.2 [M-ClO_4-2H_2O]$^+$. Anal. Cald. for $C_{25}H_{56}N_8O_4Zn_2 \cdot 3ClO_4 \cdot OH \cdot H_2O$ (996.91): C, 30.09, H, 5.92, N, 11.23, Found: C, 30.05, H, 5.91, N, 10.85. Atomic absorption spectrometry for Zn%: 13.11, Found: 13.03.

*3.5.3. 1,4-Butanediol-O,O′-[2,2′-dihydroxy-3,3′-bis(1,4,7,10-tetraazacyclododecane)] dipropyl ether dizinc(**II**) complex (**7c**)*

Colorless amorphous solid; yield: 65.3%. ^{1}H NMR (400 MHz, D_2O): δ (ppm) = 4.20–4.10 [m, 2H, 2 × NCH_2CH(OH)], 3.69–3.45 (m, 8H, 4 × OCH_2), 3.25–2.66 (m, 32H, 8 × NCH_2CH_2N; m, 4H, 2 × NCH_2CH), 1.62–1.59 (m, 4H, $CH_2CH_2CH_2CH_2$). MS (ESI): m/z = 1071.1 [M + H − 8H_2O]$^+$. Anal. Calcd. for $C_{26}H_{58}N_8O_4Zn_2 \cdot 4ClO_4 \cdot 8H_2O$ (1219.49): C, 25.58; H, 6.06; N, 9.18, Found: C, 25.33; H, 5.55; N, 8.81.

*3.5.4. Diethylene glycol-O,O′-[2,2′-dihydroxy-3,3′-bis(1,4,7,10-tetraazacyclododecane)] dipropyl ether dizinc(**II**) complex (**7d**)*

Colorless amorphous solid; yield: 71.1%. ^{1}H NMR (400 MHz, D_2O): δ (ppm) = 4.18–4.08 [m, 2H, 2 × NCH_2CH(OH)], 3.69–3.46 (m, 12H, 6 × OCH_2), 3.24–2.66 (m, 32H, 8 × NCH_2CH_2N; m, 4H, 2 × NCH_2CH). MS (ESI): m/z = 789.29 [M − 4H_2O]. $C_{26}H_{58}N_8O_5Zn_2 \cdot ClO_4 \cdot 3OH.H_2O$ (862.06). Atomic absorption spectrometry for Zn% : 15.16; Found: 15.09.

3.6. Plasmid DNA cleavage

Plasmid DNA (pUC 19) cleavage activity of complexes **7a**–**d** was monitored by using agarose gel electrophoresis. In a typical experiment, supercoiled pUC 19 DNA (10 μL, 0.025 μg/μL) in Tris–HCl buffer (100 mM, pH 7.8) was treated with different concentration of complexes **7a**–**d**, followed by dilution with the Tris–HCl buffer to a total volume of 35 μL. The samples were then incubated at 37 °C for 15 min–12 h, 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.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China (Nos. 20132020, 20372051, and 20471038), Program for New Century Excellent Talents in University, Specialized Research Fund for the Doctoral Program of Higher Education and Scientific Fund of Sichuan Province for Outstanding Young Scientist.

References

[1] (a) For reviews, see:B.N. Trawick, A.T. Daniher, J.K. Bashkin, Chem. Rev. 98 (1998) 939;
(b) D.S. Sigman, A. Mazumder, D.M. Perrin, Chem. Rev. 93 (1993) 2295;
(c) D.H. Busch, Chem. Rev. 93 (1993) 847;
(d) A.E. Martell, R.D. Hancock, Coord. Chem. Rev. 133 (1994) 39;
(e) S. Aoki, E. Kimura, Chem. Rev. 104 (2004) 769.
[2] E. Kimura, Tetrahedron 48 (1992) 6175.
[3] (a) J.J. Hagan, S.C. Taylor, M.F. Twelve, Anal. Chem. 60 (1988) 514;
(b) M.R. Spirlet, J. Rebizant, M.F. Locin, J.F. Desreux, Inorg. Chem. 23 (1984) 4278.
[4] (a) P. Caravan, J.J. Ellison, T.J. McMurry, R.B. Lauffer, Chem. Rev. 99 (1999) 2293;
(b) A. Bianchi, L. Calabi, F. Corana, S. Fontana, P. Losi, A. Maiocchi, L. Paleari, B. Valtancoli, Coord. Chem. Rev. 204 (2000) 309.
[5] (a) J.P.L. Cox, K.J. Jankowinski, R. Kataky, D. Parker, N.R.A. Beeley, B.A. Boyce, M.A.W. Eaton, K. Millar, A.T. Millican, A. Harrison, C.J. Walker, J. Chem. Soc., Chem. Commun. (1989) 797;
(b) P. Wedeking, M. Tweedle, Nucl. Med. Biol. 15 (1988) 395.
[6] E. Kimura, T. Koike, J. Chem. Soc., Chem. Commun. (1998) 1495.
[7] (a) S. Aoki, M. Shiro, T. Koike, E. Kimura, J. Am. Chem. Soc. 122 (2000) 576;
(b) B. König, M. Pelka, H. Zieg, T. Ritter, H. Bouas-Laurent, R. Bonneau, J.-P. Desvergne, J. Am. Chem. Soc. 121 (1999) 1681.
[8] E. Kikuta, T. Koike, E. Kimura, J. Inorg. Biochem. 79 (2000) 253.
[9] (a) D.D. Chand, H.J. Schneider, J.A. Angilar, F. Escartí, S.V. Lius, Inorg. Chim. Acta 316 (2001) 71;
(b) Q.-X. Xiang, H. Zhou, X.-Y. Wang, X.-Q. Yu, J.-K. Bao, R.-G. Xie, Chin. Chem. Lett. 13 (2002) 223;
(c) Q.-X. Xiang, J. Zhang, P.-Y. Liu, C.-Q. Xia, Z.-Y. Zhou, R.-G. Xie, X.-Q. Yu, J. Inorg. Biochem. 99 (2005) 1661.
[10] J.H. Kim, J. Chin, J. Am. Chem. Soc. 114 (1992) 9792.
[11] (a) A. Tsubouchi, T.C. Bruice, J. Am. Chem. Soc. 116 (1994) 11614;
(b) M. Yashiro, A. Ishikubo, M. Komiyama, J. Chem. Soc., Chem. Commun. (1995) 1793;
(c) M. Yashiro, A. Ishikubo, M. Komiyama, J. Chem. Soc., Chem. Commun. (1997) 83;
(d) R. Hettich, H.J. Schneider, J. Am. Chem. Soc. 119 (1997) 5638.
[12] (a) C. Sissi, F. Felluga, P. Tecila, F. Formaggio, M. Crisma, C. Toniolo, P. Scrimin, J. Am. Chem. Soc. 121 (1999) 4948;
(b) C. Sissi, P. Rossi, F. Felluga, F. Formaggio, M. Palumbo, P. Tecilla, C. Toniolo, P. Scrimin, J. Am. Chem. Soc. 123 (2001) 3169.
[13] F.N. Aka, M.S. Akkaya, E.U. Akkaya, J. Mol. Cat. A: Chem. 165 (2001) 291.
[14] N. Saki, E.U. Akkaya, J. Mol. Cat. A: Chem. 219 (2004) 227.
[15] B. König, M. Pelka, M. Subat, I. Dix, P.G. Jones, Eur. J. Org. Chem. (2001) 1943.
[16] A. Maxwell, M. Gellert, Adv. Protein Chem. 38 (1986) 69.
[17] G. Mouzin, H. Cousse, J.P. Rieu, A. Duflos, Synthesis (1983) 117.
[18] X.-P. Gu, I. Ikeda, M. Okahara, Synthesis (1985) 649.
[19] (a) C. Granier, R. Guilard, Tetrahedron 51 (1995) 1197;
(b) B. Boitrel, B. Andrioletti, M. Lachkar, R. Guilard, Tetrahedron Lett. 36 (1995) 4995;
(c) A.M. Groth, L.F. Lindoy, G.V. Meehan, J. Chem. Soc., Perkin Trans. 1 (1996) 1553.
[20] E. Kimura, T. Gotoh, S. Aoki, M. Shiro, Inorg. Chem. 41 (2002) 3239.
[21] (a) T.J. Atkins, J.E. Richman, W.F. Oettle, Org. Synth. 58 (1978) 86;
(b) J.E. Richman, T.J. Atkins, J. Am. Chem. Soc. 96 (1974) 2268.
[22] E. Kimura, S. Aoki, T. Koike, M. Shiro, J. Am. Chem. Soc. 119 (1997) 3068.