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Monometallic complexes of 1,4,7,10-tetraazacyclododecane containing an imidazolium side: Synthesis, characterization, and their interaction with plasmid DNA

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Abstract—The synthesis of macrocyclic polyamine monometallic complexes containing imidazolium salt groups was reported. Their interaction with pUC19 plasmid DNA was studied. The result showed that these complexes can catalyze the DNA cleavage with unprecedented reactivity under physiological conditions.

1. Introduction

The remarkable stability of the phosphodiester bond in DNA is one of the essential requirements for the survival and maintenance of life.[1] This stability is a result of the repulsion between the negatively charged phosphodiester backbone and potential nucleophiles. The half-life of the phosphodiester linkage in cleavage of DNA at pH 7.0 and 30 °C is estimated to be 200 million years.[2] A number of enzymes evolved by nature such as restriction endonucleases, recombinases, topoisomerases, and others can catalyze phosphoryl cleavage efficiently under physiological conditions.[3] However, the enzymatic reaction is exceedingly difficult to mimic in the laboratory.[4] Consequently, great efforts have been made to develop small molecule chemical nucleases that work on DNA in a similar fashion.[5] A large amount of research results show that the artificial nucleases could enhance the cleavage rates of DNA and the half-life is insignificant on the physiologically relevant time scale.[5–7] Moreover, some synthetic nucleases could also recognize and cleave specific nucleic acid structure or sequence, which have potential applications in biochemistry, molecular biology, and therapy.[8] However, the efficiency of currently available chemical nucleases is impressive yet still far from that of natural enzymes.

1,4,7,10-Tetraazacyclododecane (cyclen) has strong coordination ability toward a wide range of cations and their complexes have been widely used in DNA recognition and cleavage as chemical nucleases.[9–13] In the course of our continuing studies on the macrocyclic polyamine (cyclen) affinities toward DNA, we pay more attention to enhance the catalytic efficiency of DNA cleavage by modification of the ligands.[14–16] It was reported that a *p*-xylyl spacer plays a key role in dimeric and trimeric Zn^{II}–cyclen complex.[17] Schneider et al. found out that the cyclen ligand containing peralkylated ammonium group in the side chain has approximately the same affinity for DNA as the corresponding protonated polyamine, moreover, the positive charges can activate the phosphates to ease the liberation of leaving phosphate anion and to stabilize pretransition state complexes with nucleotides and with double-stranded DNA.[18] In addition, there were very few reports on the cyclen ligands containing imidazole group, and these ligands were limited in the synthesis and its coordination properties with metal.[19] Herein, a series of novel monometallic cyclen complexes **1a–d** containing imidazolium salt group with an *m*- and *p*-xylyl spacer (Scheme 1)

Keywords: 1,4,7,10-Tetraazacyclododecane (cyclen); Macrocyclic polyamine; Monometallic complex; Imidazolium salt; Synthesis; pUC19 DNA; Cleavage.
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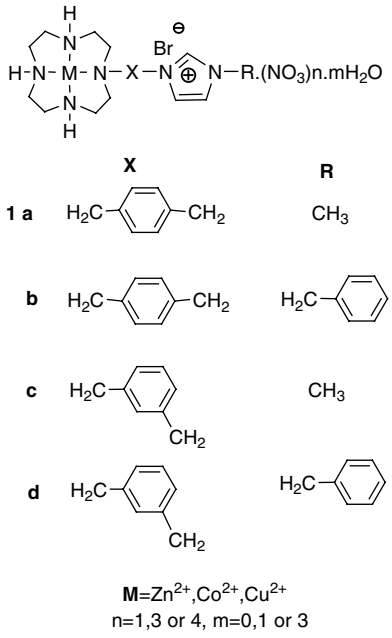


Scheme 1.

were synthesized and characterized. Their interaction with pUC19 plasmid DNA had been studied. The result shows that these complexes can catalyze the DNA cleavage with unprecedented reactivity under physiological conditions.

2. Results and discussion

2.1. Synthesis of macrocyclic polyamine mononuclear zinc(II), copper(II), and cobalt(II) complexes

The synthetic route of a series of mononuclear metal ion cyclen complexes with imidazolium salt groups is shown in Scheme 2. The monoalkylation of tri-Boc-protected cyclen **5** and *p*- or *m*-bis(bromomethyl) benzene afforded the desired product **4**, thus, a *p*- or *m*-xylyl spacer was introduced into the cyclen. By the reaction of imidazole containing N-substituted group **6** with **4**, we found the introduction of positive charges in the form of imidazolium centers in the side chains of cyclen was available with moderate yield. The Boc-protective groups were removed by adding dropwise the solution of trifluoroacetic acid (TFA) and dichloromethane. As the target chemical nucleases, macrocyclic polyamine mononuclear complexes were obtained from reacting **2** with $Zn(NO_3)_2$, $Co(NO_3)_2$ or $Cu(NO_3)_2$ in ethanol solution,[20] respectively.

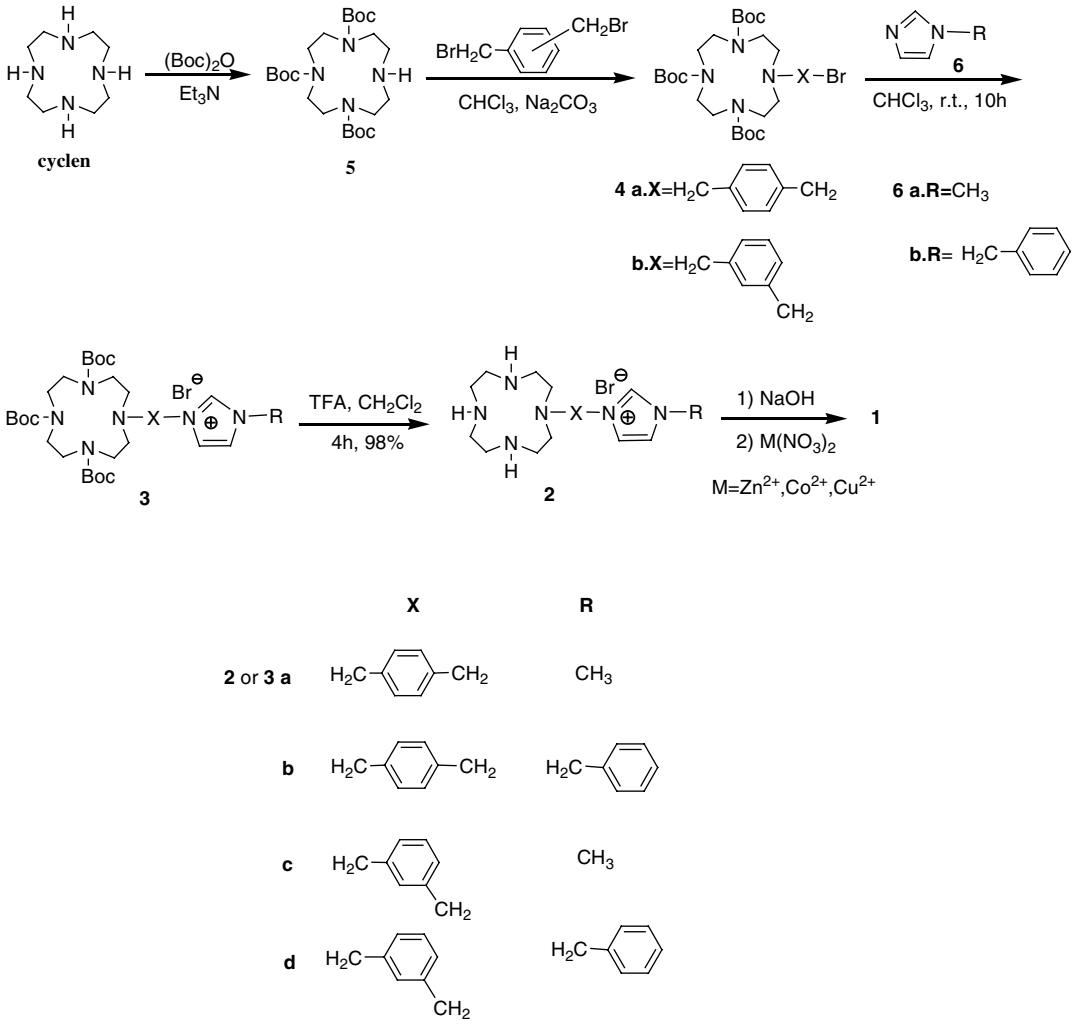
2.2. Interaction between Zn(II), Cu(II), and Co(II) complexes and plasmid pUC19 DNA

There are already some researches about the interaction of monometallic complexes and dinuclear complexes with DNA that were reported, but none of them were focused on the monometallic complexes containing imidazolium salt group. In preliminary studies, we have even investigated the positive effect of imidazole in DNA cleavage by comparing the monometallic complexes containing imidazolium salt group with free imidazole group, and the results indicated that the positively charged side chains in the complexes indeed enhance the efficiency of the catalysts.[18,21] Further, the DNA cleavage abilities of monometallic Zn(II), Cu(II), and Co(II) complexes containing imidazolium salt group were initially developed by checking the conversion of supercoiled plasmid DNA (form I) to open circular (form II) and linear (form III) DNA, and the experimental results are shown in Figures 1–4.

First we chose complex **1a**–Cu^{2+} and ascorbate to screen the conditions of DNA cleavage. Addition of ascorbic acid as reducing agent can activate the monometallic complexes and accelerate DNA nonrandom cleavage substantially by an oxidative cleavage mechanism.[21–23] Figure 1 shows the effect of concentration of ascorbate below pH 7.0 and 37 °C. It can be seen that the more concentration, the more DNA cleavage ability. And the best concentration was 0.15 mM under physiological incubation condition with 0.25 mM complex **1a**–Cu^{2+} for 180 min from lane 5 of Figure 1. Figures 2A and B show the effect of cleavage time in the complexes **1a**–Cu^{2+} and **1b**–Cu^{2+}. The reaction time varied from 0.5, 1, 2, 3 to 4 h. It is found that the longer time reacted, the more product of DNA cleavage was detected. From the data, it can also be concluded that the optimal cleavage time is 180 min for complexes **1a**–Cu^{2+} and **1b**–Cu^{2+} (0.25 mM) in lanes 6 and 11 of Figure 2.

The effect of concentration of complexes of **1a**–Cu^{2+} and **1a**–Co^{2+} is shown in the Figures 3A and B. There is an increased output of open circular (form II) DNA from super-coiled DNA with the increasing complex concentration of **1a**–Cu^{2+} or **1a**–Co^{2+} in the cleavage of pUC19 plasmid DNA (0.02 μg/μL). Significantly, under complex **1a**–Co^{2+}, form III appears in the lane 7–10 of Figure 3. And the lanes 5 and 9 of Figure 3 reveal the best conversion of complexes **1a**–Co^{2+} or **1a**–Cu^{2+} is 0.25 mM.

Figure 4 shows the effect of different complexes at 0.25 mM. The equivalent molar Zn^{2+}, Co^{2+}, Cu^{2+} complexes **1a**–**d** exhibited comparable cleavage reactivity toward DNA. It can be seen that the complex **1a**–Co^{2+} is more reactive than **1a**–Cu^{2+} and complex **1a**–Co^{2+} is more reactive than complex **1c**–Co^{2+}. It may suggest that the kind of metal ion and the steric effect of the ligands play an important role in the DNA cleavage ability. We can see that copper and zinc complex cleaves super-coiled DNA (form I) to relaxed (double-stranded cut, form II) DNA, and cobalt complexes can yield linear (single-strand broken, form III) DNA under the same conditions in the lanes 7–10 of Figures 3 and 4. Thus, the order of cleavage activity is Co(II) complex > Cu(II) complex > Zn(II) complex for metal ions, while for the ligands, **1a**–Cu^{2+} > **1c**–Cu^{2+} > **1b**–Cu^{2+} > **1d**–Cu^{2+}. The latter order is quite the reverse of the steric hindrance (CH_3– < $PhCH_2$–) and in keeping with the rigidity of the imidazolium salt-substituent group in ligands.



Scheme 2. The synthetic route of mononuclear complexes of macrocyclic polyamine with imidazolium salt groups.

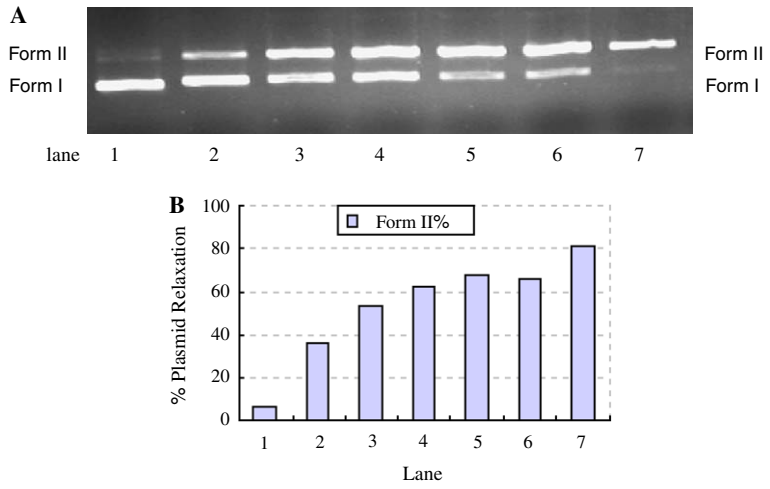


Figure 1. Effect of concentration of ascorbate on the cleavage reaction of pUC19 DNA (5 μg/ml) with complex **1a**–Cu^{2+} (0.25 mM) (ethidium bromide staining) in a Tris–HCl buffer (100 mM, pH 7.4) at 37 °C for 3 h. (A) Agarose-gel electrophoresis diagram: lane 1, DNA control and lanes 2–7, complex: **1a**–Cu^{2+}; [V_c] = 0.01, 0.05, 0.1, 0.15, 0.20, and 0.40 mM. (B) Quantitation of percentage plasmid relaxation (form II %) relative to plasmid DNA per lane.

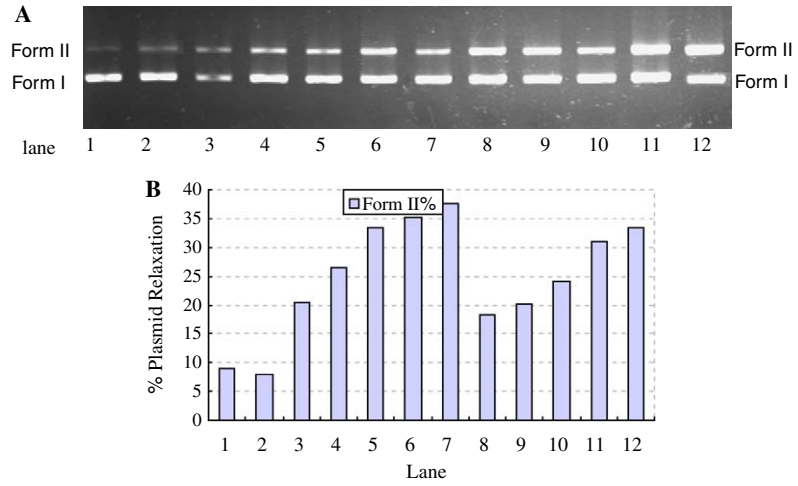


Figure 2. Effect of reaction time on the cleavage of pUC19 DNA (5 μg/ml) with copper complexes (0.25 mM) in a Tris–HCl buffer (100 mM, pH 7.4) and ascorbate (0.15 mM) at 37 °C. (A) Agarose-gel electrophoresis diagrams: lane 1, DNA control; lane 2, without complex; lanes 3–7, complex **1a**–Cu^{2+}, 0.5, 1, 2, 3, and 4 h; and lanes 8–12: complex **1b**–Cu^{2+}, 0.5, 1, 2, 3, and 4 h. (B) Quantitation of percentage plasmid relaxation (form II %) relative to plasmid DNA per lane.

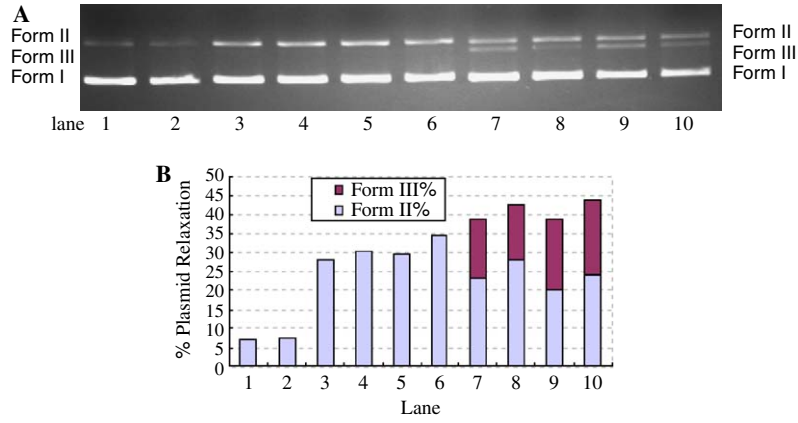


Figure 3. Effect of concentration of complexes **1a**–Cu^{2+} and **1a**–Co^{2+} in a Tris–HCl buffer (100 mM, pH 7.4) and ascorbate (0.15 mM) at 37 °C for 3 h. (A) Agarose-gel electrophoresis diagram: lane 1, DNA control; lane 2, without complex; and lanes 3–6, complex: [**1a**–Cu^{2+}] = 0.10, 0.20, 0.25, and 0.30 mM; and lanes 7–10, complex: [**1a**–Co^{2+}] = 0.10, 0.20, 0.25, and 0.30 mM. (B) Quantitation of percentage plasmid relaxation (form II % and form III %) relative to plasmid DNA per lane.

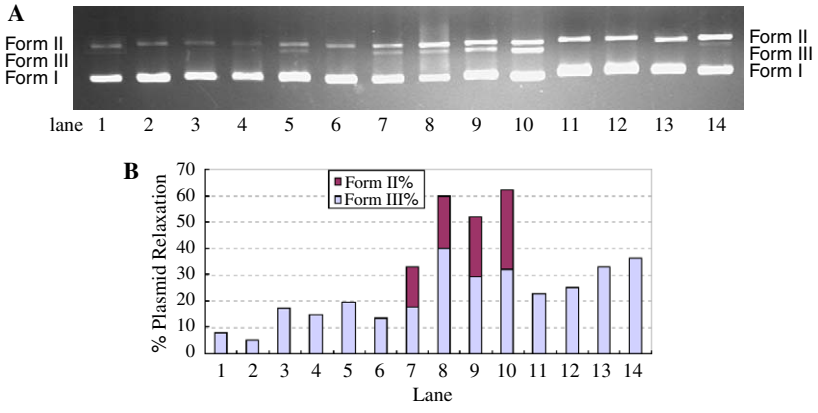


Figure 4. Effect of different complexes (0.25 mM) in a Tris–HCl buffer (100 mM, pH 7.4) and ascorbate (0.15 mM) at 37 °C for 3 h. (A) Agarose-gel electrophoresis diagram: lane 1: DNA control; lane 2: without complex; and lanes 3–14: complexes: **1d**–Zn^{2+}, **1b**–Zn^{2+}, **1c**–Zn^{2+}, **1a**–Zn^{2+}, **1d**–Co^{2+}, **1b**–Co^{2+}, **1c**–Co^{2+}, **1a**–Co^{2+}, **1d**–Cu^{2+}, **1b**–Cu^{2+}, **1c**–Cu^{2+}, and **1a**–Cu^{2+}. (B) Quantitation of percentage plasmid relaxation (form II % and form III %) relative to plasmid DNA per lane.

3. Conclusion

In this paper, we first designed and synthesized a series of novel macrocyclic polyamine ligands containing imidazolium salt group by using an *m*- or *p*-xylyl linkage, and correlated monometallic complexes **1a**–**d** were also prepared. The results of DNA cleavage show that as chemical nucleases the monometallic complexes **1a**–**d** are able to accelerate the plasmid DNA cleavage to form II nicked DNA dramatically and Co(II) complexes can cleave plasmid DNA to the linear DNA form III within shorter time in the presence of ascorbate. It suggests that positive charge led in the side chain of the complex is beneficial to activate the phosphate ester and liberate phosphate anion easily.

4. Experimental

4.1. General information

Compounds 1,4- or 1,3-bis(bromomethyl)benzene, cyclen, *N*-methyl- or *N*-benzylmethylimidazole, 1,4,7-tris(*tert*-butyloxycarbonyl)-1,4,7,10-tetraazacyclododecane (Boc_3-cyclen), and 1-bromomethyl-4- or -3-(4,7,10-tris-(*tert*-butyloxycarbonyl)-1,4,7,10-tetraazacyclododecan-1-yl-methyl)benzene were prepared as described previously.[17,24–27] Electrophoresis grade agarose and plasmid DNA (pUC19) were purchased from Takara Biotechnology Company. All other reagents were used as received. Anhydrous acetonitrile (CH_3CN), abs chloroform ($CHCl_3$), and dichloromethane (CH_2Cl_2) were distilled from calcium hydride (CaH_2). All aqueous solutions were prepared from deionized or distilled water. IR spectra were recorded on a Shimadzu FTIR-4200 spectrometer as KBr pellets or thin films on KBr plates. The ^{1}H NMR spectra were measured on a Varian INOVA-400 spectrometer (400 MHz) and the δ scale in parts per million was referenced to residual solvent peaks or internal tetramethylsilane (TMS). ESI mass spectra were performed on a Finnigan LCQ^{DECA} and high-resolution MS spectral data were recorded on a Bruker Daltonics Bio TOF. 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. Thin-layer chromatography (TLC) and column chromatography were performed with H (40–60 μ) (silica gel) and Fuji Silysia Chemical FL-100D (silica gel), respectively. TLC analysis was performed with Merck F254 silica gel-60 plates and viewed by UV light or developed with iodine stain.

4.2. General procedure for the synthesis of compounds 3a–d

N-Methyl- or *N*-benzylimidazole (0.60 mmol) was added to the solution of 1-bromomethyl-4- or -3-(4,7,10-tris-(*tert*-butyloxycarbonyl)-1,4,7,10-tetraazacyclododecan-1-yl-methyl)benzene (0.55 mmol) in 15 mL $CHCl_3$ and stirred under N_2 for 10 h. Then the reaction mixture was concentrated under reduced pressure. The remaining residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH, 9/1) to obtain **3a**–**d**.

4.2.1. Synthesis of *N*-methyl-*N*′-(4,7,10-tris(*tert*-butyloxycarbonyl)-1,4,7,10-tetraazacyclododecan-4-yl-methyl)-1-benzylimidazolium bromide (3a). As above-described synthetic method, colorless amorphous solid **3a** was obtained. Yield: 53%. R_f = 0.22 ($CHCl_3$/MeOH, 10/1). IR (KBr, cm^{-1}): 3402, 2976, 1690, 1452, 1416, 1366, 1250, 1182, 1051, 990, 850, 772, 618. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 1.42–1.46 (br, 18H, $C(CH_3)_3$), 1.56 (br, 9H, $C(CH_3)_3$), 2.62 (s, 4H, NCH_2), 3.29–3.55 (m, 12H, NCH_2), 3.72 (s, 2H, NCH_2Ar), 4.06 (s, 3H, NCH_3), 5.49 (s, 2H, $ArCH_2Ar$), 7.13 (s, 1H, imidazole-H), 7.30–7.33 (m, 4H, ArH), 7.39 (s, 2H, imidazole-H). ESI-MS: *m*/*z* = 657.6 $(M-Br)^-$, HR-MS (ESI) Calcd for $C_{35}H_{57}N_6O_6$: 657.4334. Found: 657.4342.

4.2.2. Synthesis of *N*-methyl-*N*′-(4,7,10-tris(*tert*-butyloxycarbonyl)-1,4,7,10-tetraazacyclododecan-3-yl-methyl)-1-benzylimidazolium bromide (3b). As above-described synthetic method, colorless amorphous solid **3b** was obtained. Yield: 52.9%. R_f = 0.20 ($CHCl_3$/MeOH = 10/1). IR (KBr, cm^{-1}): 3447, 2966, 1696, 1580, 1460, 1416, 1366, 1250, 1156, 979, 890, 771. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 1.31 (br, 18H, $C(CH_3)_3$), 1.47 (br, 9H, $C(CH_3)_3$), 2.57 (br, 4H, NCH_2), 3.32–3.54 (m, 12H, NCH_2), 3.72 (s, 2H, NCH_2Ar), 4.07 (s, 3H, NCH_3), 5.48 (s, 2H, $ArCH_2Ar$), 7.23 (s, 1H, imidazole-H), 7.26–7.31 (m, 4H, ArH), 7.35 (s, 2H, imidazole-H). ESI-MS: *m*/*z* = 657.3 $(M-Br)^-$, HR-MS (ESI) Calcd for $C_{35}H_{57}N_6O_6$: 657.4334. Found: 657.4339.

4.2.3. Synthesis of *N*-benzyl-*N*′-(4,7,10-tris(*tert*-butyloxycarbonyl)-1,4,7,10-tetraazacyclododecan-4-yl-methyl)-1-benzylimidazolium bromide (3c). As above-described synthetic method, colorless amorphous solid **3c** was obtained. Yield: 43.1%. R_f = 0.27 ($CHCl_3$/MeOH, 10/1). IR (KBr, cm^{-1}): 3446, 2906, 1686, 1560, 1685, 1460, 1416, 1366, 1250, 1155, 909, 850, 772. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 1.31 (br, 18H, $C(CH_3)_3$), 1.47 (br, 9H, $C(CH_3)_3$), 2.63 (br, 4H, NCH_2), 3.30–3.74 (m, 12H, NCH_2, $ArCH_2N$), 5.52 (s, 2H, $ArCH_2Ar$), 5.54 (s, 2H, $ArCH_2Ar$), 7.00 (s, 1H, imidazole-H), 7.31–7.38 (m, 4H, ArH), 7.41–7.45 (m, 7H, ArH, imidazole-H). ESI-MS: *m*/*z* = 733.4 $(M-Br)^-$, HR-MS (ESI) Calcd for $C_{41}H_{61}N_6O_6$: 733.4647. Found: 733.4637.

4.2.4. Synthesis of *N*-benzyl-*N*′-(4,7,10-tris(*tert*-butyloxycarbonyl)-1,4,7,10-tetraazacyclododecan-3-yl-methyl)-1-benzylimidazolium bromide (3d). As above-described synthetic method, colorless amorphous solid **3d** was obtained. Yield: 54.1%. R_f = 0.23 ($CHCl_3$/MeOH, 10/1). IR (KBr, cm^{-1}): 3421, 2970, 1699, 1578, 1472, 1420, 1367, 1256, 1171, 1045, 987, 866, 718, 645, 571. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 1.31 (br, 18H, $C(CH_3)_3$), 1.47 (br, 9H, $C(CH_3)_3$), 2.59 (br,4H, NCH_2), 3.30–3.55 (m, 12H, NCH_2), 3.73 (s, 2H, $ArNCH_2$), 5.52 (s, 2H, $ArCH_2Ar$), 5.57 (s, 2H, $ArCH_2$-Ar), 7.16 (s, 1H, imidazole-H), 7.34–7.42 (m, 9H, ArH), 7.47 (d, 2H, imidazole-H). ESI-MS: *m*/*z* = 733.4

$(M-Br)^-$, HR-MS (ESI) Calcd for $C_{41}H_{61}N_6O_6$: 733.4647. Found: 733.4671.

4.3. General procedure for the synthesis of compounds 2a–d·2TFA

Trifluoroacetic acid (1.05 mmol) was added dropwise to a solution of **3a**–**d** (150 mg, 0.21 mmol) in CH_2Cl_2 (10 mL) at 0 °C under N_2, and the whole mixture was stirred for 6 h at room temperature. Then the reaction mixture was concentrated under reduced pressure. And the remaining yellow oil liquid was washed three times with CH_2Cl_2 (5 mL), and we can obtain **2a**–**d**·2TFA.

4.3.1. Synthesis of *N*-methyl-*N*′-(1,4,7,10-tetraazacyclododecan-4-yl-methyl)-1-benzylimidazolium bromide (2a·2TFA). As above-described synthetic method, a yellow liquid **2a**·2TFA was obtained. Yield: 81.4%. R_f = 0.11 ($CHCl_3$/MeOH, 10/1). IR (KBr, cm^{-1}): 3444, 2978, 1754, 1662, 1567, 1430, 1416, 1368, 1256, 1160, 1045, 866, 799, 706. ^1H NMR (400 MHz, $CDCl_3$, TMS): δ = 2.54 (t, 8H, J = 8 Hz, NCH_2), 2.65 (m, 7H, NCH_2, NCH_3), 2.78 (t, 4H, J = 4 Hz, NCH_2), 3.39 (s, 3H, NH), 3.60 (s, 2H, $ArNCH_2$), 5.07 (s, 2H, $ArCH_2$-Ar), 7.06 (d, 2H, J = 8 Hz, imidazole-H), 7.27–7.38 (m, 4H, ArH), 7.53 (s, 1H, imidazole-H). ESI-MS: m/z = 357.4 $(M-Br)^-$, HR-MS (ESI) Calcd for $C_{20}H_{33}N_6$: 357.2761. Found: 357.2748.

4.3.2. Synthesis of *N*-methyl-*N*′-(1,4,7,10-tetraazacyclododecan-3-yl-methyl)-1-benzylimidazolium bromide (2b·2TFA). As above-described synthetic method, yellow liquid **2b**·2TFA was obtained. Yield: 76.1%. R_f = 0.09 ($CHCl_3$/MeOH, 10/1). IR (KBr, cm^{-1}): 3465, 2959, 1699, 1489, 1420, 1356, 1256, 1182, 1043, 997, 850, 755, 629. ^1H NMR (400 MHz, DMSO, TMS): δ = 2.71 (s, 4H, NCH_2), 2.87 (s, 4H, NCH_2), 3.08 (s, 4H, NCH_2), 3.16 (s, 4H, NCH_2), 3.74 (s, 2H, $ArCH_2N$), 3.86 (s, 3H, NCH_3), 3.96 (s, 3H, NH), 5.42 (s, 2H, $ArCH_2Ar$), 7.33–7.44 (m, 4H, ArH), 7.73 (d, 1H, J = 4 Hz, imidazole-H), 7.79 (d, 1H, imidazole-H), 9.25 (s, 1H, imidazole-H). ESI-MS: m/z = 357.6 $(M-Br)^-$, HR-MS (ESI) Calcd for $C_{20}H_{33}N_6$: 357.2761. Found: 357.2779.

4.3.3. Synthesis of *N*-benzyl-*N*′-(1,4,7,10-tetraazacyclododecan-4-yl-methyl)-1-benzylimidazolium bromide (2c·2TFA). As above-described synthetic method, yellow liquid **2c**·2TFA was obtained. Yield: 67.0%. R_f = 0.09 ($CHCl_3$/MeOH, 10/1). IR (KBr, cm^{-1}): 3434, 2964, 1680, 1551, 1454, 1414, 1351, 1200, 800, 720, 644. ^1H NMR (400 MHz, DMSO, TMS): δ = 2.53 (s, 3H, NH), 2.73 (s, 4H, NCH_2), 2.92 (s, 4H, NCH_2), 3.14 (d, 8H, J = 18 Hz, NCH_2), 3.75 (s, 2H, J = 8 Hz, $ArCH_2N$), 5.46 (s, 4H, $ArCH_2Ar$), 7.39–7.42 (m, 9H, ArH), 7.86 (s, 2H, imidazole-H), 9.54 (s, 1H, imidazole-H). ESI-MS: m/z = 357.5 $(M-Br)^-$, HR-MS (ESI) Calcd for $C_{26}H_{37}N_6$: 433.3074. Found: 433.3059.

4.3.4. Synthesis of *N*-benzyl-*N*′-(1,4,7,10-tetraazacyclododecan-3-yl-methyl)-1-benzylimidazolium bromide (2d·2TFA). As above-described synthetic method, yellow liquid **2d**·2TFA was obtained. Yield: 71.0%. R_f = 0.1 ($CHCl_3$/MeOH, 10/1). IR (KBr, cm^{-1}): 3444 3064, 2843, 1752, 1680, 1560, 1456, 1425, 1198, 1019, 961, 798, 720. ^1H NMR (400 MHz, DMSO, TMS): δ = 2.53 (s, 3H, NH), 2.76 (s, 4H, NCH_2), 3.07 (s, 4H, NCH_2) 3.22 (s, 8H, NCH_2), 3.80 (s, 2H, $ArCH_2N$), 5.49 (s, 4H, $ArCH_2Ar$), 7.38–7.47 (m, 9H, ArH), 7.88 (d, 2H, J = 6 Hz, imidazole-H), 9.53 (s, 1H, imidazole-H). ESI-MS: m/z = 433.6 $(M-Br)^-$, HR-MS (ESI) Calcd for $C_{26}H_{37}N_6$: 433.3074. Found: 433.3062.

4.4. General procedure for the synthesis of metal ion complexes 1a–d—Zn^{2+} 1a–d—Co^{2+} 1a–d—Cu^{2+}

The trifluoroacetic acid salts of ligands **2a**–**d** (0.8 mmol) were dissolved, respectively, in the 10 mL of ethanol and adjusted the aqueous solution to alkaline (pH $\geqslant$ 12) with 50% aqueous NaOH. The solutions were extracted with CH_2Cl_2 (4× 60 mL). The combined organic layer was dried overnight by anhydrous Na_2SO_4 and the solutions were concentrated to obtain a white powder **2a**–**d**. The ethanol solutions (5 mL) of **2a**–**d** were added, respectively, equimolar amount of salts $(Zn(NO_3)_2$, $Co(NO_3)_2$ or $Cu(NO_3)_2)$ in the 10 mL ethanol/water (1:1) and heated to 50 °C for 30 min. After filtration, the solids were washed with ethanol (2× 10 mL), recrystallized by ethanol/H_2O (3:1), and vacuum-dried to obtain colorless crystals twelve monometallic complexes. Complex **1c**-Zn^{2+} yield: 84.4%. IR (KBr, cm^{-1}): 3448, 3159, 2938, 2366, 1684, 1625, 1557, 1458, 1092, 856, 787, 624. ^1H NMR (400 MHz, D_2O, TMS): δ = 2.53 (s, 3H, NH), 2.91 (t, 4H, J = 10 Hz, NCH_2), 3.22 (br, 6H, NCH_2), 3.19-3.25 (br, 8H, NCH_2), 3.91 (s, 3H, NCH_3), 5.43 (s, 2H, $ArCH_2Ar$), 7.41 (d, 2H, J = 6 Hz, imidazole-H), 7.73–7.57 (m, 4H, ArH), 8.75 (s, 1H, imidazole-H). HR-MS (ESI) Calcd for $C_{20}H_{33}Cl_2N_6O_8Zn$: 619.1023. Found: 619.1038.

4.5. Plasmid DNA cleavage

The cleavage activity of complexes **1a**–**d** for plasmid DNA (pUC19) was monitored by agarose-gel electrophoresis. In a typical experiment, super-coiled pUC19 DNA (0.025 μg/μL) in Tris–HCl buffer (100 mM, pH 7.4) and ascorbate (V_c) (5 μL, 0.15 mM) were mixed with 8 μL complexes **1a**–**d** and followed by dilution with the Tris–HCl buffer to a total volume of 40 μL. The sample was then incubated at 37 °C for 30–240 min 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 by the estimation of the intensity of the DNA bands using a gel documentation system.

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