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Chiral multinuclear macrocyclic polyamine complexes: Synthesis, characterization and their interaction with plasmid DNA

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Abstract—A series of multinuclear macrocyclic polyamine metal (Zn^{2+}, Cu^{2+}, Co^{2+}) complexes containing chiral dipeptide linkage were synthesized and used as artificial nuclease enzyme model. The interaction between the complexes and plasmid DNA (pUC19) was studied, and the results revealed that these complexes could act as powerful catalysts for the cleavage of plasmid DNA under physiological conditions.

1. Introduction

With the high abilities in recognizing specific DNA sequence and catalyzing the hydrolysis of phosphate diester bonds, chemical nucleases have rapidly become an invaluable research tool in the fields of biology, bioorganic chemistry, therapy, and molecular biology.[1,2] Many complexes have been designed and studied as artificial enzyme models. In the commonly used ligands, 1,4,7,10-tetraazacyclododecane (cyclen) has a special structure and can coordinate with most metal cations (transition metal ions and lanthanide ions[3–6]). Therefore, metal–cyclen complexes were widely used in DNA recognition,[7,8] cleavage,[9] and enzyme mimics.[10–13]

Many nucleases carry one or more metal centers. In most cases, synergistic effects exist between the metal centers.[14] It has been reported that Zn(II) is a key metal ion for the development of artificial metallonucleases. Barton and coworkers proved that a series of mononuclear Zn(II) complexes, which bind to peptide chains tethered to a rhodium complex, could act as intercalator and promote DNA cleavage. Scrimin and coworkers synthesized dinuclear Zn^{2+} complexes and used the complexes as artificial nucleases, they proved that these complexes were more effective in the cleavage of RNA and DNA under physiological conditions than mononuclear complex, which revealed that a high degree of synergistic effect existed between the two metal ion centers.[15–17] Suh and coworkers reported that Cu(II) or Co(III) complexes of polyamines could be used as catalytic drugs to promote the selective cleavage of protein.[18,19] The heteroatoms on amino acid or peptide side chains and backbone could enhance the ability of ligands to coordinate with metal ions.[20] Many dinuclear metal complexes were studied as enzyme models,[2,21–23] however, to the best of our knowledge, the reports of studies of multinuclear metal complexes with chiral peptide linkage and their uses in the cleavage of DNA remain rare. Due to the folded structure of the peptide, the two catalytic centers might have an appropriate distance and angle in the catalytic process. In this paper, we synthesized a series of multinuclear metal complexes with chiral dipeptide spacers and used them as artificial nuclease to study their interaction with plasmid DNA (pUC19).

Keywords: 1,4,7,10-Tetraazacyclododecane (cyclen); Macrocyclic polyamine; Multinuclear complexes; DNA cleavage; Chemical nuclease.
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2. Results and discussion

2.1. Synthesis

The synthetic route of these multinuclear ligands is shown in Scheme 1. The *Z*-(benzylacetate) group was introduced to be an effective protecting group for the carboxyl acid on side chain. Compounds **4** were obtained by the reaction between **2** and *Z*-protected amines **3** in the presence of (DCC/HOBt). The desired products were purified by silica gel column chromatography to avoid the loss from washing by citric acid and aqueous $NaHCO_3$ solution. Deprotected products **5** were obtained by catalytic method under H_2 atmosphere with Pd/C. Subsequent preparation was processed through the traditional way of peptide synthesis (DCC/HOBt). Complexes **7a** and **7b** were obtained in around 65–74% yields. The Boc protective groups were removed by adding the solution of trifluoroacetic acid (TFA) dropwise in dichloromethane. Multinuclear metal complexes were obtained by adding the excessive salt [$Zn(ClO_4)_2$, $Co(NO_3)_2$ or $Cu(NO_3)_2$] solution under refluxing conditions for 2 h. All new compounds and metal complexes were confirmed by analytic methods.

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

The interaction of dinuclear metal complexes with pUC19 was studied by monitoring the conversion of circular supercoiled DNA (Form I) to nicked (Form II) and linear (Form III) DNA. The amount of strand scission was assessed by agarose gel electrophoresis.

Frist, we choose the complexes **8a**-M (M = Zn, Cu, or Co) as the catalyst to optimize the conditions of DNA cleavage. Different metal complexes displayed different catalytic effect, the results are shown in Figure 1. Among the three complexes, **8a**-Co is the best catalyst for DNA cleavage. When we increase the concentration of complexes from 0.14 to 0.28 mM, only **8a**-Co showed obvious increase of catalytic effect, the conversion of Form I increased from 40% to 51%. On the contrary, the catalytic ability of **8a**-Zn and **8a**-Cu was slightly decreased with the raise of concentration. Our subsequent efforts focused on the reactivity of complex **8a**-Co under physiological conditions.

Figures 2 and 3 show the effects of reaction time and concentration of complex in the cleavage reaction catalyzed by **8a**-Co. The results revealed that dinuclear macrocyclic polyamine complexes as chemical nucleases are capable to accelerate the plasmid DNA dramatically. Increasing the reaction time resulted in the increase of catalytic ability. But when extending the reaction time to 72 h, the amount of Form II was slightly decreased. So the best interaction time is 48 h. Figure 3 indicated that the best concentration of complex **8a**-Co is 0.028 mM. Further increase of concentration led to the decrease of Form II. The reason might be that the part of DNA was incised to pieces in high concentration of catalyst.

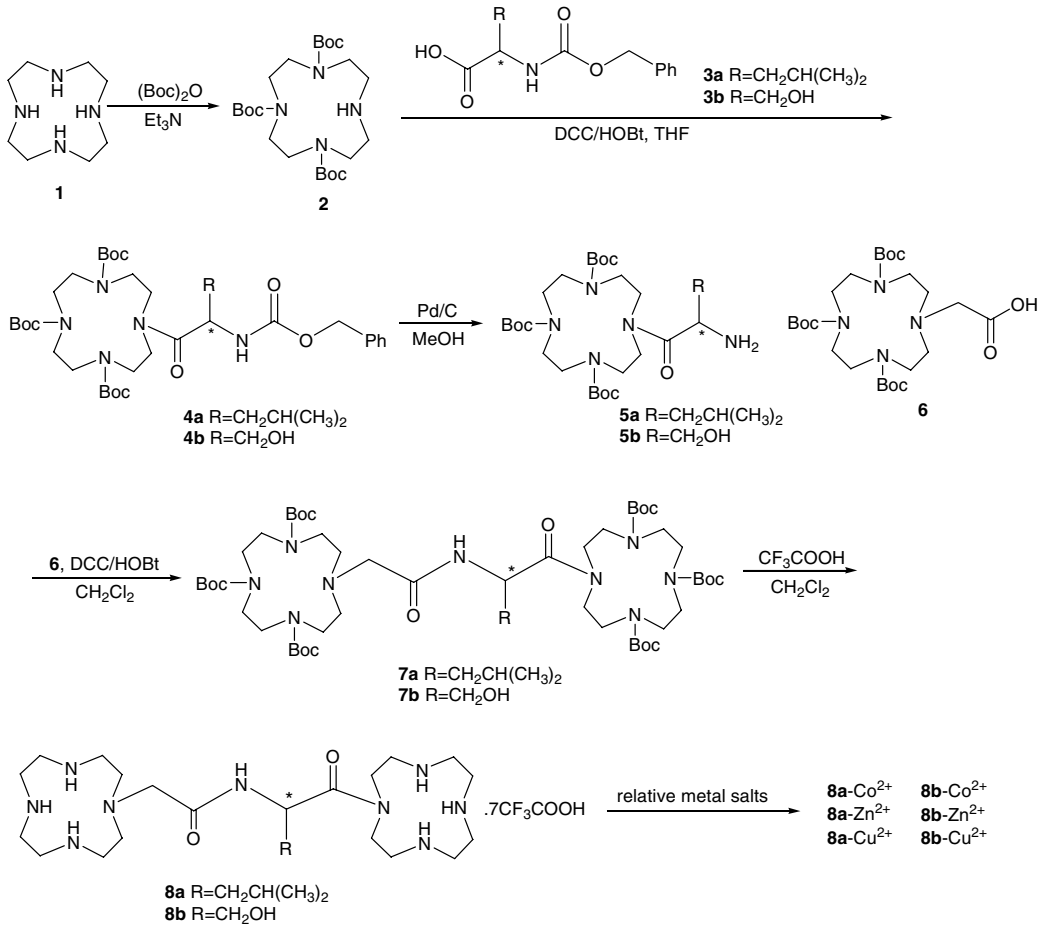
Then we studied the complexes **8b**-M. Unlike **8a**-M, **8b**-Cu instead of **8b**-Co was the best catalyst for DNA cleavage. A solution containing plasmid DNA was incubated in a 0.5 mL tube with catalyst **8b**-Zn, **8b**-Cu and **8b**-Co (0.14 mM) in a Na_2HPO_4/NaH_2PO_4 buffer (100 mM, pH 7.0) under 37 °C for 48 h. The amount of nicked DNA (Form II) was 37.37%, 60.57%, and 31.31%, respectively. So, our subsequent efforts focused on the catalytic ability of complex **8b**-Cu under physiological conditions. Figures 4 and 5 show the effects of reaction time and concentration of catalyst in **8b**-Cu-catalyzed cleavage reaction. The results showed that complex **8b**-Cu was an excellent chemical nuclease and the amount of nicked DNA (Form II) was more than the reaction catalyzed by **8a**-Co. On the other hand, similar to **8a**-Co, the catalytic ability of **8b**-Cu was increased in association with the raise of reaction time or concentration of complex. However, when we increased the two factors to a special level, the linear DNA (Form III) appeared.

By comparing the results of reactions catalyzed by the complexes derived from **8a** and **8b**, we can conclude that the peptide linkage did play an important role in the DNA cleavage process. The structure of the bridge spacer may influence the character of the complexes. When using serine to build the spacer, the hydroxyl group might change the coordinative property, and, as a result, the metal complexes derived from **8b** showed different catalytic ability from those derived from **8a**. In the catalytic cleavage process, Cu^{2+} was chosen to be the most suitable metal ion for **8b**, while Co^{2+} was most suitable for **8a**. The results of parallel experiments are shown in Figure 6. The amine group might also have the ability to promote the performance of metal complex in the cleavage of DNA under physiological conditions. Meanwhile, due to the special folded structure of the peptide, the two catalytic centers might have an appropriate distance and angle in the catalytic process, so a synergistic effect might exist in the system.

3. Experimental

3.1. General information

All reagents were purchased from commercial sources and used without further purification. Anhydrous acetonitrile (CH_3CN), chloroform ($CHCl_3$), dichloromethane (CH_2Cl_2), and tetrahydrofuran (THF) were dried and purified under nitrogen by using standard methods and were distilled immediately before use. All aqueous solutions were prepared from deionized or distilled water. Electrophoresis grade agarose and plasmid DNA (pUC19) were purchased from Takara Biotechnology Company. MS (ESI) mass spectral data were recorded on a Finnigan LCQ^{DECA} mass spectrometer. HRMS spectral data were recorded on Bruker Daltonics Bio TOF. ^1H NMR spectral data were measured on a Varian INOVA-400 spectrometer and chemical shifts in ppm are reported relative to internal Me_4Si ($CDCl_3$). Elemental analyses were performed using a Carlo-Elba 1106 elemental analytical instrument. IR spectra were



Scheme 1. Preparation of chiral multinuclear macrocyclic polyamine complexes.

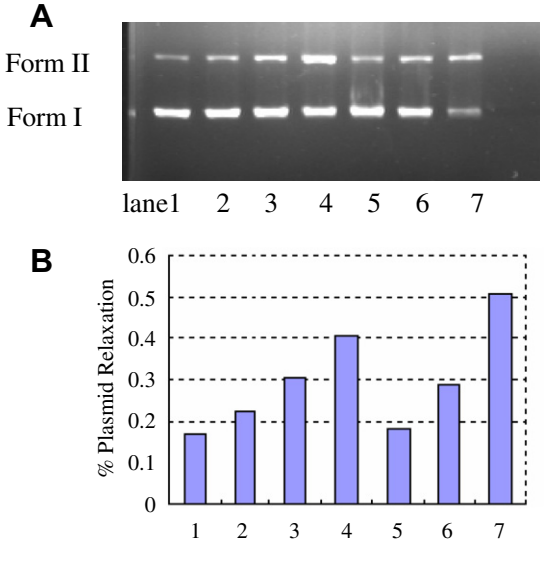


Figure 1. Cleavage reaction of pUC19 DNA (5 μg/mL) in Tris–HCl buffer (100 mM, pH 7.4) catalyzed by different metal complexes at 37 °C for 52 h. (A) Diagram of agarose gel electrophoresis. Lane 1, DNA control; lane 2, 0.14 mM **8a**-Zn^{2+}; lane 3, 0.14 mM **8a**-Cu^{2+}; lane 4, 0.14 mM **8a**-Co^{2+}; lane 5, 0.28 mM **8a**-Zn^{2+}; lane 6, 0.28 mM **8a**-Cu^{2+}; lane 7, 0.28 mM **8a**-Co^{2+}. (B) Quantitation of % plasmid relaxation relative to plasmid DNA per lane.

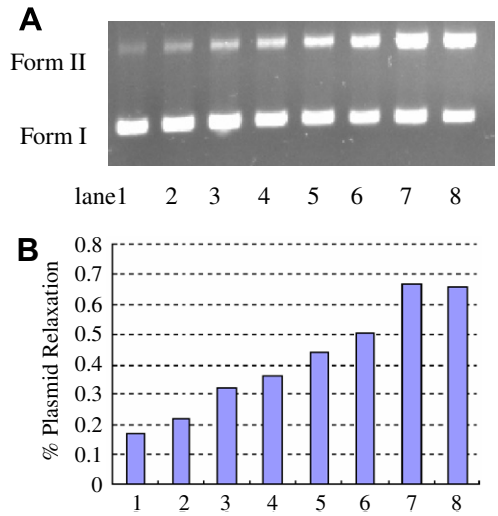


Figure 2. Effect of reaction time on the cleavage of pUC19 DNA (5 μg/mL) with **8a**-Co (0.14 mM) in a Na_2HPO_4/NaH_2PO_4 buffer (100 mM, pH 7.0) at 37 °C. (A) Agarose gel electrophoresis diagrams: lane 1, DNA control; lanes 2–8, complex **8a**-Co, 1, 2, 4, 8, 12, 24, 48, and 72 h. (B) Quantitation of % plasmid relaxation (Form II) relative to plasmid DNA per lane.

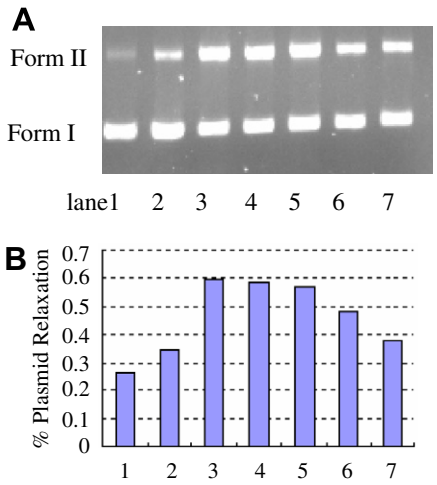


Figure 3. Effect of concentration on the cleavage of pUC19 DNA (5 μg/mL) with **8a**-Co in a Na_2HPO_4/NaH_2PO_4 buffer (100 mM, pH 7.0) and at 37 °C for 48 h. (A) Agarose gel electrophoresis diagrams: lane 1, DNA control; lanes 2–7, 0.014, 0.028, 0.070, 0.14, 0.28, and 1.14 mM. (B) Quantitation of % plasmid relaxation relative to plasmid DNA per lane.

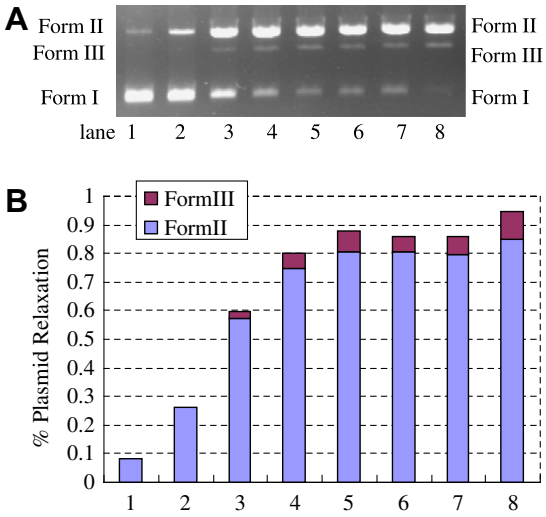


Figure 5. Effect of concentration on the cleavage of pUC19 DNA (5 μg/mL) with **8b**-Cu in a Na_2HPO_4/NaH_2PO_4 buffer (100 mM, pH 7.0) at 37 °C for 72 h. (A) Agarose gel electrophoresis diagrams: lane 1, DNA control; lanes 2–8, 0.007, 0.014, 0.028, 0.07, 0.14, 0.28, 0.57. (B) Quantitation of open circular form (Form II) and linear form (Form III) relative to plasmid DNA per lane.

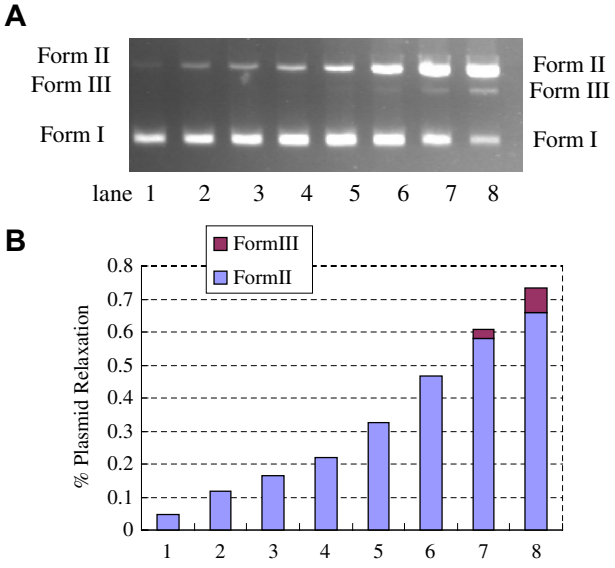


Figure 4. Effect of reaction time on the cleavage of pUC19 DNA (5 μg/mL) with **8b**-Cu (0.14 mM) in a Na_2HPO_4/NaH_2PO_4 buffer (100 mM, pH 7.0) at 37 °C. (A) Agarose gel electrophoresis diagrams: lane 1, DNA control; lanes 2–8, complex **8a**-Cu, 1, 2, 4, 8, 12, 24, 48, and 72. (B) Quantitation of % plasmid relaxation open circular form (Form II) and linear form (Form III) relative to plasmid DNA per lane.

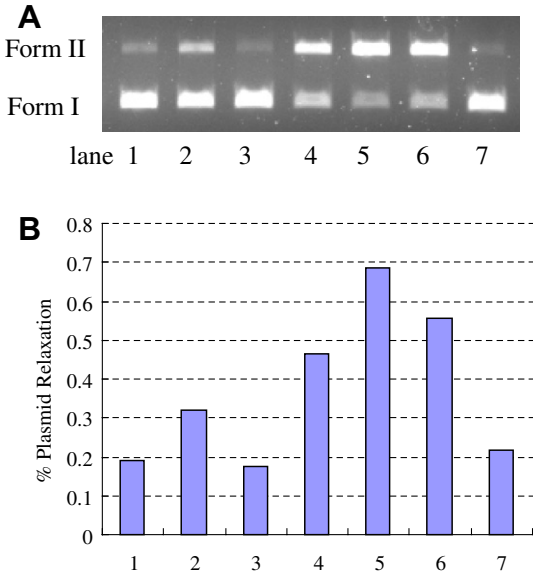


Figure 6. Effect of different complexes on the cleavage of pUC19 DNA (5 μg/mL) in a Na_2HPO_4/NaH_2PO_4 buffer (100 mM, pH 7.0) at 37 °C for 24 h. (A) Agarose gel electrophoresis diagrams: lane 1, DNA control; lanes 2–7, 0.28 mM $Cu(NO_3)_2$, 0.28 mM $Co(NO_3)_2$, 0.14 mM **8a**-Cu, 0.14 mM **8b**-Cu, 0.14 mM **8a**-Co, 0.14 mM **8b**-Co. (B) Quantitation of open circular form (Form I) and linear form (Form II) relative to plasmid DNA per lane.

recorded on a Shimadzu FTIR-4200 spectrometer as KBr pellets or thin films on KBr plates. Melting points were determined using a micro-melting point apparatus without any corrections. Cyclen and 1,4,7-tris(*tert*-butyloxycaronyl)-1,4,7,10-tetraazacyclododecan[24,25] were prepared as previously reported. The compound **6** (4,7,10-tris(*tert*-butoxycarbonyl)-1,4,7,10-tetraazacyclododecan-1-yl) acetic acid was synthesized according to the literature.[26]

3.2. General procedure for the synthesis of compounds 4

Compounds **4** were synthesized by the following general method. A THF solution of **3** (4.22 mmol), 3Boc-cyclen (4.22 mmol), and 1-hydroxybenzotriazole-1-hydroxybenzotriazole (HOBt) (5.06 mmol) was cooled to 0 °C in an ice bath. *N*,*N*′-dicyclohexylcarbodiimide (DCC) (5.06 mmol) was dissolved in 20 mL THF and added

dropwise to the solutions. The mixture was stirred at 0 °C for 2 h and warmed to room temperature, and stirred at room temperature overnight. The suspension was filtered and the precipitate was washed twice with small amount of cold THF. The filtrate was purified by silica gel column chromatography (EtOAC/hexane = 1:2) to give colorless amorphous solid **4**.

Compound **4a**, colorless amorphous solid. Yield: 78%. IR (KBr, cm^{-1}): 3421, 2974, 2931, 1696, 1647, 1466, 1411, 1366, 1250, 1164, 1043, 966, 864, 775. ^1H NMR (400 MHz, $CDCl_3$, TMS) $\delta = 7.33$ (m, 5H, Ph–H); 5.44 (d, $J = 8.8$ Hz, 1H, N–H); 5.07 (s, 2H, $–CH_2–Ph$); 4.63 (t, $J = 8.4$ Hz, 1H, CH); 3.49 (m, 16H, $–CH_2$); 1.74–1.76 (m, 2H, $–CH_2$); 1.45–1.48 (m, 27H, $OC(CH_3)_3$); 1.01 (d, $J = 6.4$ Hz, 3H, $–CH_3$); 0.93 (d, $J = 6.8$ Hz, 3H, $–CH_3$). HRMS-ESI of $C_{37}H_{61}N_5O_9$: 742.4361 $[M+Na]^+$. Found: 742.4349 $[M+Na]^+$.

Compound **4b**, colorless amorphous solid. Yield: 72%. IR (KBr, cm^{-1}): 3437, 2975, 2931, 1687, 1639, 1466, 1413, 1366, 1250, 1164, 1019, 966, 858, 777. ^1H NMR (400 MHz, $CDCl_3$, TMS) $\delta = 7.26–7.37$ (m, 5H, Ph–H); 5.73 (d, $J = 7.6$ Hz, 1H, N–H); 5.12 (s, 2H, $–CH_2–Ph$); 4.69–4.74 (m, 1H, –OH); 3.39–3.80 (m, 15H, $–CH_2$); 2.65 (s, 4H, $–CH_2$); 1.44–1.48 (m, 27H, $OC(CH_3)_3$). HRMS-ESI of $C_{34}H_{55}N_5O_{10}$: 716.3841 $[M+Na]^+$. Found: 716.3868 $[M+Na]^+$.

3.3. General procedure for the synthesis of compounds 5

A 50 mL two-necked round-bottomed flask was charged with the solid of Pd/C (0.14 g) under N_2 atmosphere, then the solution of **4** (1.9 mmol) in MeOH was injected. The mixture was stirred for 4 h under H_2 atmosphere at room temperature. After filtration, the filtrate was evaporated off and purified by silica gel column chromatography ($CHCl_3$/MeOH = 9:1).

Compound **5a**, colorless amorphous solid. Yield: 79%. IR (KBr, cm^{-1}): 3433, 2974, 2931, 1695, 1647, 1467, 1411, 1366, 1249, 1164, 966, 787. ^1H NMR (400 MHz, $CDCl_3$, TMS) $\delta = 3.17–3.75$ (m, 17H, –CH, $–NCH_2$); 1.78–1.86 (m, 3H, $–CH_2–CH$); 1.47 (m, 27H, $OC(CH_3)_3$); 0.94–0.97 (m, 6H, $–CH_3$). HRMS-ESI of $C_{29}H_{55}N_5O_7$: 586.4174 $[M+H]^+$. Found: 586.4189 $[M+H]^+$.

Compound **5b**, colorless amorphous solid. Yield: 80%. IR (KBr, cm^{-1}): 3420, 2975, 2930, 1696, 1467, 1412, 1367, 1249, 1162, 1108, 1055, 978, 847, 787. ^1H NMR (400 MHz, $CDCl_3$, TMS) $\delta = 3.97$ (t, $J = 7.2$ Hz, 1H, –CH); 3.79–3.83 (m, 1H, –OH); 3.37–3.71 (m, 20H, $–CH_2$, $–CH_2N$); 1.45–1.46 (m, 27H, $OC(CH_3)_3$). HRMS-ESI of $C_{26}H_{49}N_5O_8$: 560.3654 $[M+H]^+$. Found 560.3650 $[M+H]^+$.

3.4. General procedure for the synthesis of compounds 7

To a solution of **6** (1.30 mmol) in CH_2Cl_2 was added DCC (1.56 mmol), then **5** (1.30 mmol) in CH_2Cl_2 and HOBt (1.56 mmol) were added at 0 °C and the mixture was stirred for 2 h at same temperature, and then stirred at room temperature for 24 h under N_2 atmosphere. The reaction mixture was filtered, the filtrate was dried, and the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (**7a**: EtOAc/hexane = 2:1; **7b**: EtOAc/hexane = 4:1).

Compound **7a**, colorless amorphous solid. Yield: 67%. IR (KBr, cm^{-1}): 3448, 2974, 2930, 1687, 1465, 1413, 1366,1250, 1165, 781.^1H NMR (400 MHz, $CDCl_3$, TMS) $\delta = 4.88–4.92$ (m, 1H, –NH); 3.21–3.53 (m, 30H, $–CH_2$); 2.84 (s, 4H, $–CH_2$); 1.69 (m, 3H, $CH–CH_2$); 1.44–1.48 (m, 54H, $OC(CH_3)_3$); 0.98 (d, $J = 6.4$ Hz, 3H, CH_3); 0.92 ($J = 6.4$ Hz, 3H, CH_3). HRMS-ESI of $C_{54}H_{99}N_9O_{14}$: 1120.7204 $[M+Na]^+$. Found: 1120.7185 $[M+Na]^+$.

Compound **7b**, colorless amorphous solid. Yiled: 65%. IR (KBr, cm^{-1}): 3447, 2975, 2930, 1699, 1465, 1414, 1366, 1249, 1165, 776. ^1H NMR (400 MHz, $CDCl_3$, TMS) $\delta = 4.92$ (d, $J = 4.8$ Hz, 1H, NH); 3.73–3.81 (m, 5H, CH_2CH); 3.26–3.54 (m, 26H, CH_2); 2.78 (s, 4H, CH_2); 1.81 (s, 2H, CH_2OH). ESI-MS of $C_{51}H_{93}N_9O_{15}$: 1094.7 $[M+Na]^+$. Found 1094.7 $[M+Na]^+$.

3.5. General procedure for the synthesis of compounds 8

Trifluoroacetic acid (0.66 mmol) was added dropwise to a solution of **7** (0.11 mmol) in CH_2Cl_2 (5 mL) at 0 °C under N_2 atmosphere. After stirring overnight at room temperature, the reaction mixture was concentrated under reduced pressure below 40 °C to give the crude product. And the resulting yellow solid was recrystallized from anhydrous ether and washed three times with anhydrous ether (5 mL).

Compound **8a**. Yield: 84.0%. ^1H NMR (400 MHz, D_2O) $\delta = 4.72–4.74$ (m, 1H, –CH); 3.58–3.63 (m, 2H, $–CH_2$); 3.21–3.34 (2H, $–CH_2$); 3.04–3.12 (m, 4H, $–CH_2$); 1.69–1.77 (m, 2H, CH_2); 1.56–1.59 (m, 1H, –CH); 0.99–1.01 (m, 6H, CH_3). HRMS-ESI of $C_{24}H_{51}N_9O_2$: 498.4238 $[M+H]^+$. Found: 498.4229 $[M+H]^+$.

Compound **8b**. Yield: 82%. ^1H NMR (400 MHz, D_2O) $\delta = 4.11–4.15$ (m, 1H, –CH); 3.83–3.94 (m, 4H, $–CH_2$); 3.66–3.69 (m, 2H, $–CH_2$); 3.48–3.56 (m, 2H, $–CH_2$); 3.26–3.27 (m, 12H, $–CH_2$); 3.16–3.21 (m, 8 H, $–CH_2$); 3.01 (s, 8H, CH_2). HRMS-ESI of $C_{21}H_{45}N_9O_3$: 472.3718 $[M+H]^+$. Found: 472.3710 $[M+H]^+$.

3.6. General procedure for the preparation of metal complexes 8-Zn, 8-Co, and 8-Cu

The trifluoroacetic acid salts of ligand **8** (0.9 mmol) were dissolved in 5 mL of water and adjusted the aqueous solution to alkaline (pH $\geqslant$ 12) with 50% aqueous NaOH. The solutions were extracted with CH_2Cl_2 (4×10 mL). Then organic layer was dried over anhydrous Na_2SO_4 and the solutions were concentrated under reduced pressure to give yellow oil. To the ethanol solutions (5 mL) of **8** was added excessive salt [$Zn(ClO_4)_2$, $Co(NO_3)_2$ or $Cu(NO_3)_2$] solution in 10 mL of ethanol and stirred overnight under room temperature and then refluxed for 2 h. The solution

was concentrated under reduced pressure, the solid was purified by centrifugal device, and washed with ethanol (2×10 mL).

8a-Co^{2+}: Anal. Calcd for $C_{24}H_{51}N_9O_2{\cdot}3Co(NO_3){\cdot}3(OH)$ (911.19): C, 31.62; H, 5.97; N, 18.44. Found: C, 31.27; H, 5.94; N, 18.38. Atomic absorption spectrometry for Co%: 19.40. Found: 19.78. IR (KBr, cm^{-1}): 3433, 2924, 2854, 1627, 1562, 1472, 1384.

8a-Zn^{2+}: Anal. Calcd for $C_{24}H_{51}N_9O_2{\cdot}2Zn(ClO_4)_2{\cdot}2Zn(OH)_2{\cdot}5H_2O$ (1314.98): C, 21.92; H, 4.98; N, 9.58. Found: C, 22.10; H, 4.74; N, 9.45. Atomic absorption spectrometry for Zn%: 19.89. Found: 19.84. IR (KBr, cm^{-1}): 3450, 2927, 1639, 1460, 1143, 1120, 1088, 627.

8a-Cu^{2+}: Anal. Calcd for $C_{24}H_{51}N_9O_2{\cdot}2Cu(OH)_2{\cdot}2Cu(NO_3)_2$ (1067.10): C, 26.99; H, 5.19; N, 17.05. Found: C, 26.97; H, 4.91; N, 16.12. Atomic absorption spectrometry for Cu%: 23.80. Found: 23.66. IR (KBr, cm^{-1}): 3438, 3231, 2933, 1621, 1384, 1359, 1073.

8b-Co^{2+}: Anal. Calcd for $C_{21}H_{45}N_9O_3{\cdot}3Co(NO_3){\cdot}3(OH)$ (885.14): C, 28.48; H, 5.46; N,18.98. Found: C, 29.43; H, 5.32; N, 19.23. Atomic absorption spectrometry for Co%: 19.97. Found: 19.78. IR (KBr, cm^{-1}): 3430, 2927, 2879, 1620, 1578, 1384.

8b-Zn^{2+}: Anal. Calcd for $C_{21}H_{45}N_9O_3{\cdot}Zn(ClO_4)_2{\cdot}Zn(ClO_4)(OH)\ C_2H_5OH$ (963.11): C, 28.66; H, 5.44; N, 13.08. Found: C, 28.31; H, 5.04; N, 12.77. Atomic absorption spectrometry for Zn%: 13.57. Found: 13.54. IR (KBr, cm^{-1}): 3436, 2921, 2867, 1639, 1452, 1117, 1108, 619.

8b-Cu^{2+}: Anal. Calcd for $C_{21}H_{45}N_9O_3{\cdot}2Cu(OH)_2{\cdot}2Cu(NO_3)_2$ (1041.87): C, 24.21; H, 4.74; N, 17.48. Found: C, 24.57; H, 4.71; N, 16.67. Atomic absorption spectrometry for Cu%: 24.40. Found: 25.05. IR (KBr, cm^{-1}): 3432, 3243, 2933, 1633, 1420, 1384, 1347, 1074.

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