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PAPER

Steric effects on the catalytic activities of zinc(II) complexes containing [12] aneN$_3$ ligating units in the cleavage of the RNA and DNA model phosphates†

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A series of *N*-methylated mono- and di-[12]aneN$_3$ ligands (**5–9**) have been synthesized and characterized. The steric effects on the catalytic activities of their mononuclear and dinuclear zinc(II) complexes in the cleavage of a RNA model 2-hydroxypropyl 4-nitrophenyl phosphate (HPNPP, **3**) and a DNA model methyl 4-nitrophenyl phosphates (MPNPP, **4**) in methanol have been investigated at 25 °C. In the cleavage of phosphate **3** catalyzed by the mononuclear complexes, derived from the *N*-methylation in the [12]aneN$_3$ backbone, the plots of k_{obs} *versus* [Zn(II)] changed from an upward curvature to linearity with increasing level of methylation, indicating that *N*-methylations led to a reduction of dinuclear association that was responsible for the synergetic effect. Compared to the activities of the complex with non-methylated di-[12]aneN$_3$ ligand, those of the dinuclear zinc(II) complex (Zn_2-**8**), which has the two *N*-methyl groups, were reduced by two orders of magnitude as measured by the second-order rate constants and synergetic effect in the cleavage of both model compounds. For reactions catalyzed by the fully *N*-methylated dinuclear complex (Zn_2-**9**), no synergetic effect was observed. Nevertheless, complex Zn_2-**8** still showed the remarkable catalytic efficiency, with rate accelerations of 10^{9-10}-fold in the cleavage of each of the two phosphates relative to the background reactions, and the synergetic effects of up to 561 folds. pH jump experiments confirmed that the rate-limiting step in the cleavage of **3** by Zn_2-**8** involved the binding process, while that in the reaction with **4** was the chemical cleavage of the P–O bond. Steric effects in the cleavage reactions were analyzed in detail and were compared with the electronic effect caused by oxy anion bridging group in the di-[12]aneN$_3$ ligand and also with the hydrophobic effect observed in other systems. The work has further confirmed that the combination of the cooperativity between two metal ions and a medium effect could result in excellent catalytic activities for the cleavage of phosphate diesters.

Introduction

The development of synthetic agents able to hydrolytically cleave phosphate diester bonds with high efficiency is a fascinating challenge and has received considerable interest during the last two decades.[1–7] These studies will provide several benefits, such as shedding light on understanding of how catalysis works under biological conditions and their potential applications as artificial restriction enzymes for molecular biology which would be highly valuable in the manipulation of DNA, and developing the anti-DNA drugs. Inspired by the structures of natural nucleases, much work has focused on the synthesis of dinuclear or polynuclear metal complexes.[8–11] Dinuclear system can contribute to the cleavage of a phosphate diesters in the following four ways: (1) by double Lewis acid activation of a phosphate; (2) through bifunctional catalysis in which the metal ions activate the phosphate and supply a metal-coordinated hydroxide acting as nucleophile or base; (3) as an electrostatic reservoir of positive charge to interact with the anionic phosphate to stabilize the transition state for the phosphoryl transfer reaction; and (4) by assisting the departure of the phosphate's leaving group through coordination.

Recently, the Brown group has studied the systems comprising mono- and dinuclear-Zn(II) complexes of 1,5,9-triazacyclododecane ([12]aneN$_3$, **1**, Scheme 1) and 1,3-bis N$_1$-(1,5,9-triazacyclododecyl)propane (di-[12]aneN$_3$, **2**) in alcohols and found that the dinuclear complex Zn_2-**2** can promote the cleavage of a

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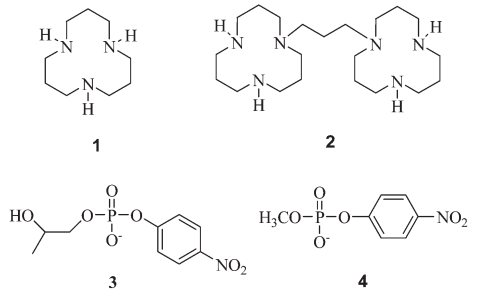
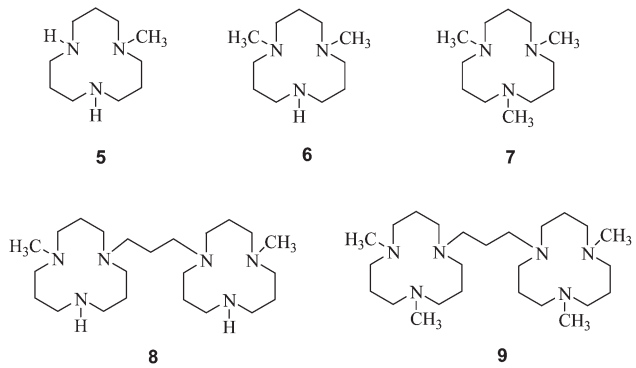
†Electronic supplementary information (ESI) available: Tables of kinetic data and method for triflate ion inhibition, plots of kinetics data as a function of metal complexes concentrations and triflate, NMR and mass spectroscopic data for the identity of ligands **5–9**, and the intermediates. See DOI: 10.1039/c2ob25624j



Scheme 1 Structures of mono-, di-[12]aneN$_3$ ligands **1–2** and model phosphate diesters **3–4**.



Scheme 2 Structures of *N*-methylated mono- and di-[12]aneN$_3$ ligands.

well-studied RNA model, namely 2-hydroxypropyl *p*-nitrophenyl phosphate (HPNPP, **3**) and DNA model methyl *p*-nitrophenyl phosphate (MPNPP, **4)** by a spectacular factor of up to $10^{12–14}$-fold, when compared to the background reactions.[12,13] They further explored almost every aspect of the catalytic cleavage of the phosphate diesters with the dinuclear Zn(II) complexes of di-[12]aneN$_3$ ligands. In particular, they varied the structures of the catalysts by using different metal ions and different linker structures (with various lengths and electronic properties), various RNA and DNA model substrates, and different reaction mediums (including methanol, ethanol, and aqueous ethanol).[14–21] Their work sheds light on the catalytic mechanism of the natural nucleases and provides rich information for preparing enzyme mimics. However, the steric effects of the catalyst structures have not been investigated in detail, this also plays very important roles in understanding reaction mechanisms and controlling reactions.

Here we report on the preparation of a series of mononuclear and dinuclear zinc(II) complexes with ligands **5–9** (Scheme 2), which bear different numbers of *N*-methyl groups, and their catalytic performance in the cleavage of phosphates **3** and **4** in methanol. The incorporation of *N*-methyl groups in the [12]-aneN$_3$ backbone clearly reduced the catalytic activity of the corresponding metal complexes, especially the synergistic effect between the two metal ions. The steric effects of *N*-methylations in the [12]aneN$_3$ ligands on the catalytic cleavage of phosphates have been analyzed in detail in this work.

Experimental

Materials

Methanol (99.8% anhydrous), sodium methoxide (0.5 M solution in methanol), perchloric acid (70% aqueous solution), zinc triflate, and tetrabutylammonium triflate were purchased from Aldrich and used as supplied. 1,3-Dibromopropane, formaldehyde, formic acid, sodium hydroxide, concentrated hydrochloric acid, sodium borohydride, acetonitrile, ethanol, and chloroform used in the experiments were purchased from Beijing Chemical Reagents Company.

Synthesis

Syntheses of 2-hydroxypropyl-*p*-nitrophenyl phosphate (HPNPP, **3**),[22] methyl-*p*-nitrophenyl phosphate (MPNPP, **4**),[23] octahydro-3a,6a,9a-triazaphenalene (a precursor of [12]aneN$_3$, **10**),[24] 1-methyl-1,5,9-triazacyclododecane (**5**),[25] 1,5-dimethyl-1,5,9-triazacyclododecane (**6**),[24] and 1,5,9-trimethyl-1,5,9-triazacyclododecane (**7**)[26] were synthesized according to the literature procedures and characterized by ^{1}H-NMR.

Preparation of ligands 8 and 9

Ligand 8. To a solution of [12]aneN$_3$ precursor **10** (3.22 g, 17.8 mmol) in 120 mL of dry acetonitrile was added dropwise 1,3-dibromopropane (1.79 g, 8.88 mmol) in 5 mL of dry acetonitrile with stirring. The mixture was refluxed in an oil bath maintained at 98 °C over 60 h to give a beige precipitate, which was collected *via* filtration, washed with cold acetonitrile and ether, and dried at room temperature under vacuum. This afforded 3.7 g of the dibromide salts **11** with a yield of 74%. This product was subsequently used as a precursor for ligands **8** and **9**.

Compound **11** (1.46 g, 2.59 mmol) was dissolved in 100 mL of absolute ethanol and placed in an ice-bath. Then 0.3 g (7.9 mmol) of $NaBH_4$ was slowly added to the above solution, and the mixture was stirred overnight under nitrogen. After quenching the reaction mixture with 1 M HCl, the solvent was removed by rotary evaporation under reduced pressure. To the residue was added 20 mL of 10 M NaOH solution and the mixture was stirred at room temperature for 2 h. The free ligand **8** was extracted with chloroform, dried with sodium sulfate, and finally evaporated under reduced pressure to afford the yellow oil 0.94 g with a yield of 88%. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 2.66–2.64 (t, 8H), 2.45–2.38 (m 12H), 2.36–2.33 (m 4H), 2.32–2.28 (m, 4H), 2.10 (s, 6H), 1.61–1.54 (m, 14H). ^{13}C NMR (101 MHz, $CDCl_3$, ppm): δ 57.6, 54.3, 54.0, 51.6, 50.1, 49.1, 48.8, 41.5, 25.2, 25.2, 24.8, 23.2. IR (KBr, cm^{-1}): 3304 (s), 3227 (s), 2956 (s), 2687 (s), 2656 (s), 1612 (m), 1586 (w), 1487 (m), 1464 (m), 1420 (m), 1307 (w), 1267 (w), 1140 (w), 1089 (w), 1066 (w), 1015 (w), 923 (w), 745 (w), 668 (w), 578 (w). HR-MS (*m*/*z*) found (calcd) for $C_{23}H_{51}N_6$ (M): $[M + H]^+$, 411.4172 (411.4175).

Ligand 9. Compound **11** (2.03 g, 3.60 mmol) obtained *via* the above process was added to 20 mL of a mixture of formaldehyde, hydrochloric acid, and formic acid (in a 10 : 1 : 10 volume ratio). After refluxing for 48 h and the evaporation of

water, the residue was mixed with 10 mL of concentrated HCl, the resulted white precipitates was collected and washed with cold ethanol and diethyl ether, and subsequently dried under vacuum, which afforded the HCl salt of **9**. The free ligand **9** was obtained by base neutralization with 10 M NaOH, 1.34 g, yield 85%. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 2.62 (m, 8H), 2.50 (m, 16H), 2.36–2.32 (t, 4H), 2.25 (s, 12H), 1.68–1.61 (m, 14H). ^{13}C NMR (101 MHz, $CDCl_3$, ppm): δ 53.0, 52.9, 52.0, 49.2, 43.2, 22.3, 22.1. IR (KBr, cm^{-1}): 3430 (s), 2959 (s), 2639 (s), 1683 (m), 1633 (m), 1486 (s), 1461 (s), 1302 (w), 1257 (w), 1227 (w), 1152 (w), 1111 (m), 1068 (m), 1047 (m), 1020 (m), 969 (w), 921 (m), 906 (m), 881 (w), 760 (m), 739 (m), 591 (w), 499 (w). HR-MS (*m*/*z*) found (calcd) for $C_{25}H_{55}N_6$ (M): $[M + H]^+$, 439.4486 (439.4488).

Preparation of the stock solutions of the zinc(II) complexes

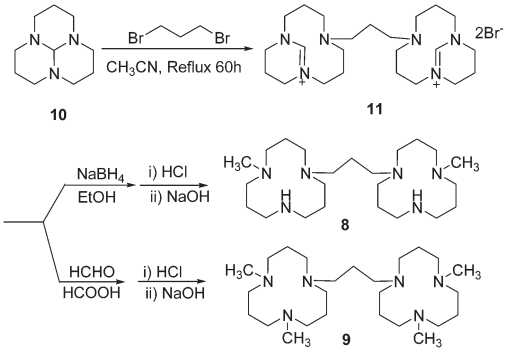
For the mononuclear complexes, the stock solutions were prepared *in situ* at a ${}^{s}_{sp}pH$[27] equal to the corresponding ${}^{s}_{sp}pK_a$ in methanol by addition of aliquots of stock solutions of sodium methoxide, *N*-methylated-1,5,9-triazacyclododecanes (**5–7**) and $Zn(CF_3SO_3)_2$ in a 0.5 : 1 : 1 molar ratio. No effects attributable to the order of addition of the complex components were observed in this case. The dinuclear complexes Zn_2-**8** and Zn_2-**9** were prepared as a 2.5 mM solution in methanol according to the procedure established in the previous literatures.[12] This involved the sequential addition of aliquots of stock solutions of sodium methoxide, dinuclear ligand (**8** or **9**), and $Zn(CF_3SO_3)_2$ such that the relative amounts were of a 1 : 1 : 2 molar ratios, respectively. The solution being kept for 50 min before the kinetic measurement were recorded in order to ensure that the dinuclear complexes had fully formed.

Kinetics

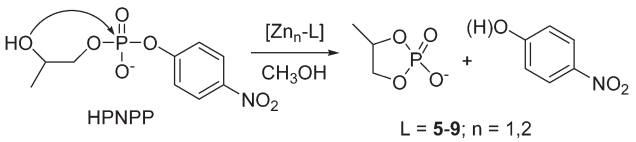
The rates of cleavage of HPNPP (0.04 mM) catalyzed by the mononuclear complexes Zn-**L** (**L** = **5–7**) and dinuclear complex Zn_2-**8** and Zn_2-**9** as well as that of methanolysis of MPNPP catalyzed by complexes Zn-**5** and Zn_2-**8** were followed by monitoring the appearance of *p*-nitrophenol at 320 nm using a Cary 300 UV-vis spectrophotometer with the cell compartment thermostated at 25.0 ± 0.1 °C. Second order rate constants for the methoxide reactions of **3** and **4** were taken from literature as (2.56 ± 0.16) × 10^{-3} M^{-1} s^{-1}[22] and (7.9 ± 0.6) × 10^{-7} M^{-1} s^{-1}[28] at 25 °C.

The dependence of the methanolysis rate on base concentration of methyl *p*-nitrophenyl phosphate (MPNPP, **4**) catalyzed by Zn_2-**8** was studied by adding aliquots of stock solutions of sodium methoxide or perchloric acid to the cell containing 0.4 mM of the [Zn_2-**8**] complex. The reaction was initiated immediately by the addition of MPNPP solution so that the final substrate concentration in the UV cell was 4 × 10^{-5} M. In general, the reactions followed good first-order kinetics up to about three half-times of the methanolysis reaction. The pseudo-first order rate constants (k_{obs}) were determined by an NLLSQ fitting of the absorbance *versus* time traces to a standard exponential model.

The rates of the cleavage of HPNPP (0.04 mM) catalyzed by the Zn_2-**8** complex were determined using an Applied Photophysics SX-17MV stopped-flow reaction analyzer thermostated at 25.0 °C. Reactions were followed by monitoring the rate of loss of the starting material at 280 nm and appearance of the product *p*-nitrophenol at 320 nm. The study of the base dependence of the cleavage of HPNPP catalyzed by Zn_2-**8** was performed by "pH jump" experiments where one syringe of the stopped-flow reaction analyzer contained a 0.4 mM solution of Zn_2-**8** and another syringe contained a 0.08 mM solution of HPNPP in methanol along with the required amount of perchloric acid or sodium methoxide.



Scheme 3 Synthesis of ligands **8** and **9**.



Scheme 4 Cleavage of HPNPP catalyzed by metal complexes of **5–9**.

Results and discussion

Synthesis of ligands 8 and 9

The synthesis of new ligands **8** and **9** was straightforward. The preparation began with the alkylation reactions between the [12]aneN$_3$ precursor **10** and 1,3-dibromopropane (Scheme 3), which produced bicyclic amidinium salts **11** as the key intermediates. The reduction of the intermediate **11** with $NaBH_4$, further acid hydrolysis and base neutralization yielded ligand **8**, which contains di-*N*-methyl groups. Through the Eschweiler–Clarke reaction, *e.g.*, the reaction of **11** with a mixture of formaldehyde and formic acid, with subsequent acid hydrolysis and neutralization, tetra-*N*-methylated ligand **9** was obtained in good yield. The two new ligands were fully characterized with ^{1}H-NMR, ^{13}C-NMR, IR and HR-MS (see ESI†).

In the ^{1}H-NMR spectra, the protons from methylene groups of [12]aneN$_3$ backbone appear around 1.60, 2.50, and 2.80 ppm as multiplets, which are consistent with those reported in the literatures.[29] The *N*-methyl groups on the [12]aneN$_3$ unit appear as singlet at 2.06 and 2.25 ppm for ligand 8 and 9, respectively.[24]

Catalytic cleavage of HPNPP

The catalytic activities of the metal complexes with ligands **5–9** on the cleavage of HPNPP (Scheme 4) were investigated in

methanol. To avoid the effect of triflate counter ions for the fast reactions with Zn_2-**8**, the kinetic data were corrected for the triflate inhibition constants (see ESI†).

(i) Cleavage of 3 with mononuclear zinc(II) complexes. Fig. 1 shows the plots of the observed pseudo-first order rate constants for the cleavage of HPNPP **3** as a function of [Zn-**L**] (**L** = **5**, **6**, **7**) in the presence of 0.5 equivalent of CH_3O^- to set the ^s_spH of the solution at self-buffered conditions.

For ligands **5** and **6**, their plots show upward curvatures, indicating a bimolecular process which are similar to that of mononuclear complexes with [12]aneN$_3$ ligands in the literatures.[12] By fitting the plots in the eqn (1), it affords k_2^{obs} and k_3^{obs} for Zn-**5** and Zn-**6**, respectively.

$$k_{obs} = k_2^{obs}[Zn - L] + k_3^{obs}[Zn - L]^2 \quad (1)$$

For Zn-**7**, the plot is almost linearity, indicating that a bimolecular process is prevented due to the steric effect from the three *N*-methyl groups in the ligand backbone.

All of the kinetic data is listed in Table 1 for comparison. It can be seen that *N*-methylations of the [12]aneN$_3$ unit gradually reduced the catalytic activity of their corresponding mononuclear species. These reductions were not too great, since the k_2 values are very close for all three mononuclear complexes. However, the incorporation of *N*-methyl groups greatly affects the synergistic effect among the mononuclear species. Zinc(II) complexes with ligands bearing mono- or di-*N*-methyl groups (**5** and **6**, respectively) can still associate to bring bimolecular process. Meanwhile, the presence of tri-*N*-methyl groups in the [12]aneN$_3$ unit resulted in no synergetic effect at all. It is noteworthy that the k_2 values are also similar to those of zinc(II) complexes of [12]aneN$_3$ ligands containing *N*-benzyl or *N*-(3-benzyl-1,2,3-triazolyl)methyl moieties in our previous work.[30]

(ii) Cleavage of 3 with dinuclear zinc(II) complexes. In the preliminary work, the reaction conditions were optimized by varying equivalents of base (sodium methoxide), zinc(II) ion, and by adjusting the mixing time. The same conclusion was reached as the literature reported:[12,13] the most reactive catalysts were obtained *in situ* through sequential addition of stock solutions of sodium methoxide, ligand, and Zn(OTf)$_2$ to anhydrous methanol such that [CH_3O^-] : [**8**/**9**] : [Zn(II)] = 1 : 1 : 2.

The dinuclear complex Zn_2-**8** with one equivalent of added methoxide produces a catalyst for the cleavage of **3**, which has remarkable activity in methanol. Fig. 2 includes a plot of k_{obs} *vs.* [CH_3O^-] = [Zn_2-**8**] determined at a measured ^s_spH of 9.2 ± 0.1. The rapid rate of the reaction requires it to be monitored by stopped-flow spectrophotometry at 320 nm, which is the wavelength corresponding to the appearance of *p*-nitrophenol. After correction for the triflate inhibition, the plot of k_{obs}^{corr} *versus* [Zn_2-**8**] is linear, the second order rate constant (k_2) evaluated from the gradient of the linear plot is (3.03 ± 0.098) × 10^3 M^{-1} s^{-1}. It can be seen that the k_2 value exhibited by Zn_2-**8** is 1.18 × 10^6-fold greater than that for the methoxide-promoted cleavage of **3** (k_2^{OMe} = 2.56 × 10^{-3} M^{-1} s^{-1}). The synergetic effect between two [12]aneN$_3$ units is 561-fold when compared to those of Zn-**5** and Zn-**6**.

For the reaction of **3** with Zn_2-**9**, the kinetic data show that the rate constants (k_{obs}) *versus* concentration exhibit a linear relationship. The corresponding k_2 value of (4.35 ± 0.06) M^{-1} s^{-1} for

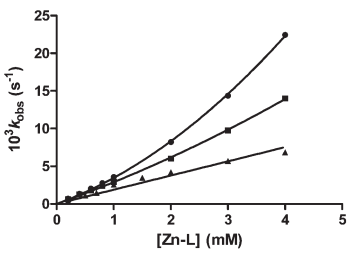


Fig. 1 Plots of k_{obs} *vs.* [Zn-L] (L = **5** ●, **6** ■, **7** ▲) for the cleavage of HPNPP (4 × 10^{-5} M) at [–OCH_3]/[Zn(II)] = 0.5, ^s_spH = 9.7 ± 0.1 for Zn-**5**, ^s_spH = 9.5 ± 0.1 for Zn-**6**, and ^s_spH = 9.4 ± 0.1 for Zn-**7** at 25.0 ± 0.1 °C.

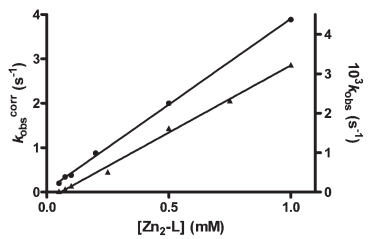


Fig. 2 Plots of k_{obs} *vs.* [Zn_2-L] (L = **8** ▲, **9** ●) for the cleavage of HPNPP (4 × 10^{-5} M) in the presence of 1 eq. of added CH_3O^- per complex giving ^s_spH = 9.2 ± 0.1 for Zn_2-**8** (left *Y* axis) and ^s_spH = 9.4 ± 0.1 for Zn_2-**9** (right *Y* axis), at 25 ± 0.1 °C.

Table 1 Kinetic constants for the cleavage of **3** mediated by zinc(II) complexes of ligand **5–9** at 25.0 ± 0.1 °C

Cat.	^s_spH	k_2 (M^{-1} s^{-1})	$k_3 \times 10^{-3}$ (M^{-2} s^{-1})	Synergetic effect	Catalytic acceleration at self-buffered ^s_spH
$^-$OMe	—	2.56 × 10^{-3}[a]	—		
Zn-**1**	9.1 ± 0.1	18.9[a]	1.8 ± 0.4[a]		3.4 × 10^8
Zn-**5**	9.7 ± 0.1	2.7 ± 0.1	0.71 ± 0.03		1.2 × 10^7
Zn-**6**	9.5 ± 0.1	2.70 ± 0.08	0.20 ± 0.02		2.0 × 10^7
Zn-**7**	9.4 ± 0.1	1.88 ± 0.08	—		1.7 × 10^7
Zn_2-**2**	9.5 ± 0.1	(2.75 ± 0.10) × 10^5[a]	—	7230	2.0 × 10^{12}
Zn_2-**8**	9.2 ± 0.1	(3.03 ± 0.08) × 10^3	—	561	4.4 × 10^{10}
Zn_2-**9**	9.4 ± 0.1	4.35 ± 0.06	—	1.1	4.0 × 10^7

[a] See ref. 12.

this reaction is almost double that of Zn-**7**, indicating that there is no significant synergetic effect between the two metal ions.

Catalytic cleavage of MPNPP

As a DNA model phosphate, the cleavage of MPNPP **4** is much slower when it is compared with the cleavage of **3**. In the case of **3**, the cleavage of the *p*-nirophenolate group is actually an intramolecular ring closure which is more than 10^3-fold faster than that of **4**.[17] A nucleophile species such as hydroxide or alkoxide is not directly involved in the cleavage of **3**. Meanwhile in the case of **4**, the leaving group *p*-nitrophenole is replaced by a nucleophile through intermolecular or catalyst-mediated attack. In order to obtain information about the steric effect on the cleavage of **4**, the kinetic data were measured in the presence of metal complexes Zn-**5** and Zn_2-**8**.

(i) Cleavage of 4 with Zn-5. The methanolysis of **4** catalyzed by Zn-**5**is very slow. The rate constants of the reaction were obtained from the initial rate procedure by fitting the first 5-10% of the absolute *versus* time traces by linear regression and then converting to first order rate constants (k_{obs}) by dividing them by the expected absorbance change, if the reaction were to reach 100% completion, as reported in the literature.[17] The plot of the k_{obs} *versus* [Zn-**5**] is shown in Fig. 3A, which has linearity dependence. The second rate constant is $k_2 = (4.1 \pm 0.2) \times 10^{-4}$ M^{-1} s^{-1}, corresponding to an enhancement of approximately 520-fold, when compared to that of methoxide.

(ii) Cleavage of 4 with Zn_2-8. For the methanolysis of **4** with Zn_2-**8**, the kinetics were measured by monitoring the entire process using UV spectroscopy. The plot of k_{obs} *versus* [Zn_2-**8**] shows some evidence of saturation binding, similar to the catalytic behavior of Zn_2-**2**. However, after correction of the effect of the weaker triflate inhibition, the plot becomes almost linear, which is difficult to fit with a standard Michaelis–Menten kinetics as for that of Zn_2-**2**. The linear fit of the plot afforded a second rate constant as $(1.78 \pm 0.04) \times 10^{-1}$ M^{-1} s^{-1}, which is 2.2×10^5-fold greater than that of methoxide-promoted cleavage of **4**, the synergetic effect between the [12]aneN$_3$ units is approximately 217-fold when compared with that of Zn-**5**. In comparison with Zn_2-**2**, *N*-methylations in complex Zn_2-**8** clearly reduced the catalytic activity by approximately 620-fold in terms of the k_2 values (see Table 2).

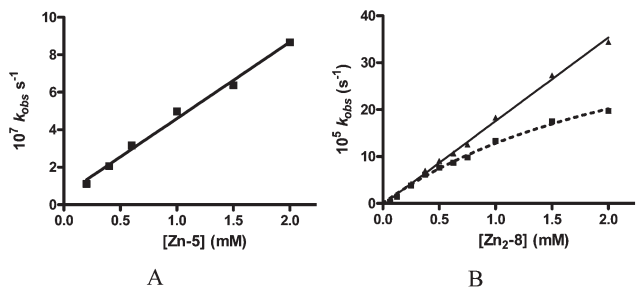


Fig. 3 (A) Plot of k_{obs} *vs.* [Zn-**5**] for the cleavage of MPNPP (4×10^{-5} M) at ^s_spH 9.8 ± 0.1 and 25 ± 0.1 °C. (B) Plot of k_{obs} *vs.* [Zn_2-**8**] for the cleavage of MPNPP (4×10^{-5} M) at ^s_spH 9.1 ± 0.1 and 25 ± 0.1 °C. The dotted line is presented as a visual aid directed through all actual data points (■); the solid line is a linear fit through the data corrected for inhibition by the triflate counterions (▲).

In the multi-step mechanism of the catalytic cleavage of DNA model **4**, the rate determining step was proposed to be the third step, *i.e.*, the chemical cleavage of 4-nitrophenole from **4**,[13] which often resulted in the saturation kinetics. The different concentration dependencies shown by Zn_2-**8** (linear behavior) and by Zn_2-**2** (saturation behavior) may result from the weaker binding strength between **4** and Zn_2-**8** due to the steric effect from the *N*-methyl groups, which suggests that the saturation binding was not reached in the studied concentration range of Zn_2-**8**. For this reason, pH dependencies of the kinetics in the cleavage of both phosphates catalyzed by Zn_2-**8** were investigated, which supports the known mechanism.

pH jump experiments for the reactions of 3 and 4 with Zn_2-8

In the cleavage of **3** and **4** with Zn_2-**2** in methanol, the reactions follow the same mechanism, which includes two binding steps and one chemical cleavage step. However, the rate-limiting step with **3** is its binding process while that with the slower reacting **4** is the chemical step of cleavage of the bound substrate to release *p*-nitrophenol. These have been clearly revealed by their kinetics of the concentration dependencies. In the case of Zn_2-**8**, the kinetics of the concentration dependence for the reactions with **3** and **4** are both in linearity, making it difficulty to clarify the rate-limiting step. Thus the influence of pH on the reactions was checked by varying the base amount in the catalytic system by using the pH-jump procedure. The kinetic data are plotted in Fig. 4. It can be seen that the k_{obs} for the cyclization of **3** decreased as the methoxide ratio was increased, but it increased as the quantity of base was reduced. The methanolysis of **4** shows a bell-shaped ^s_spH/rate profile.

The results are completely consistent with those found for the Zn_2-**2** system (see Scheme 5). For the reaction with HPNPP **3**, the increase in rate with added acid is consistent with a process depending on binding the substrate to the complex devoid of an associate methoxide with its higher net positive charge attracting

Table 2 Kinetic data for the cleavage of **4** mediated by Zn-**5** and Zn_2-**8** at 25.0 ± 0.1 °C

Cat	^s_spH	k_2 (M^{-1} s^{-1})	Synergetic effect	Catalytic acceleration at self-buffered ^s_spH
$^-$OMe	—	$(7.9 \pm 0.6) \times 10^{-7}$[a]		—
Zn-**5**	9.7 ± 0.1	$(4.1 \pm 0.2) \times 10^{-4}$		6.0×10^6
Zn_2-**2**	9.5 ± 0.1	110 ± 27[b]	—	2.6×10^{12}
Zn_2-**8**	9.2 ± 0.1	$(1.78 \pm 0.04) \times 10^{-1}$	217	8.3×10^9

[a] See ref. 28. [b] See ref. 12.

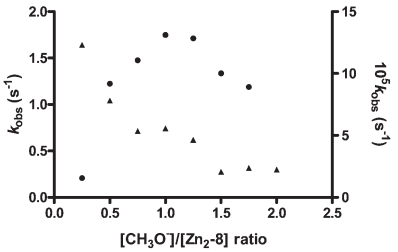
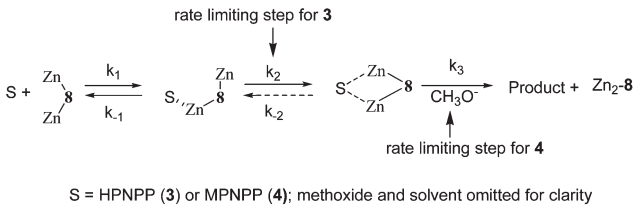


Fig. 4 Plots of the observed pseudo-first order rate constants for the methanolysis of 4 × 10^{-5} M HPNPP (▲, left axis) catalyzed by 3.2 × 10^{-4} M Zn_2-**8** or 4 × 10^{-5} M MPNPP (●, right axis) catalyzed by 1 × 10^{-3} M Zn_2-**8** as a function of the [CH_3O^-]/[Zn_2-**8**] ratio at 25 ± 0.1 °C.



S = HPNPP (**3**) or MPNPP (**4**); methoxide and solvent omitted for clarity

Scheme 5 Proposed mechanism for the cleavage of **3**/**4** promoted by Zn_2-**8**.

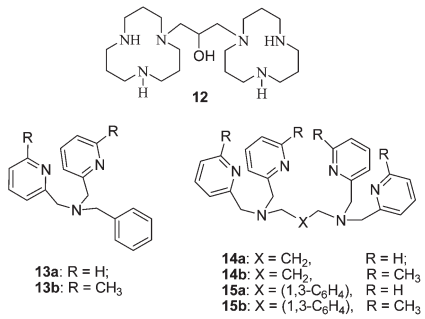
the negatively charged HPNPP. The chemical step of methoxide-dependent cyclization (k_3) is faster than the rearrangement step (k_2). For the reaction with MPNPP **4**, the maximum rate occurs when the [CH_3O^-]/[Zn_2-**8**] ratio is unity, which clearly indicates that the process requires the combination of substrate binding to Zn(II) ion and a basic role involving methoxide. Thus the rate limiting step in the catalytic cleavage of HPNPP is the binding process, and that for slow reacting MPNPP is the chemical cleavage of the P–O bond.

Analysis of the steric effect on the catalytic cleavage of phosphates

It is apparent that the incorporation of *N*-methyl groups in the [12]aneN$_3$ backbone clearly impairs the catalytic efficiency in the cleavage of RNA and DNA model phosphates **3** and **4**. However, the extent of this effect varies among the different reaction systems.

For the cleavage of **3** with Zn:**L** (**L** = **5**, **6**, **7**), the *N*-methylations has little effect on the second order rate constant k_2 values as they are 2.7, 2.7, and 1.88 M^{-1} s^{-1}, respectively, for the three mononuclear complexes. However, their association ability to form the cooperative dinuclear species was greatly decreased along the increase of the number of *N*-methyl groups due to the steric effect. The values of k_3 are decreased by 2.1 and 9-fold when one and two *N*-methyls are incorporated into [12]aneN$_3$. For the complex containing fully *N*-methylated ligand (Zn-**7**), there is almost no upward curvature in the plot of k_{obs} *vs.* [Zn]. In comparison with the reactivity of methoxide, the activities of these mononuclear complexes are still significant, their second order rate constants are approximately 1000 times higher than that of methoxide.

For the dinuclear complexes, the steric effects are more obvious. Compared to those of Zn_2-**2**, the second order rate constant for the cleavage of **3** by Zn_2-**8**, is reduced by 91-fold. Meanwhile the synergetic effect between the two [12]aneN$_3$ units is reduced by 13-fold. For the reactions with **4** catalyzed by Zn_2-**8**, it was found that the second order rate constant is reduced by 620-fold relative to that of Zn_2-**2**.



Scheme 6 Structures of ligand **13**–**15**.

The electronic effect caused by the oxyanion bridging group in Zn_2-**12** (Scheme 6) reduced the second order rate constant for the cleavage of HPNPP to be 7.6 M^{-1} s^{-1} and thus caused a 3700-fold rate reduction.[19] In contrast, the presence of di-*N*-methyl groups in Zn_2-**8** caused only a 91-fold reduction of activity. However, the presence of tetra-*N*-methyls in Zn_2-**9** resulted in 63 000 times less activity in terms of the k_2 value. Thus the steric effects in the di-[12]aneN$_3$ systems acuminated exponentially.

Another noteworthy point is that the steric effect has to be analyzed carefully. It was found that the addition of methyl substituents in ligands **13**–**15** (Scheme 6) provided a rate enhancement in comparison with those of the non-substituted ligands for the cleavage of **3**.[31,32] For mononuclear zinc(II) complexes of **13a** and **13b**, a factor of 4 rate enhancement was observed at the self-buffered conditions for methylated ligand **13b**. In the dinuclear systems, complex Zn_2-**14b** was 10^3 times more reactive than complex Zn_2-**14a**, and complex Zn_2-**15b** was 10 times more reactive than Zn_2-**15a**. These enhancements have been attributed to the decrease of local polarity due to the hydrophobic property of methyl group. In the case of ligand **5**–**9**, the *N*-methylations directly affect on the coordinating nitrogen donor, which clearly weakens the ligand binding to the metal ions as well as the complex binding to the substrate, both aspects result in poor catalysis.

Nevertheless, Zn_2-**8** is still among the most active catalysts for the cleavage of the DNA and RNA model phosphates. The second order rate constants in the methanolysis of HPNPP and MPNPP are respectively 1.18 × 10^6-fold and 2.2 × 10^5-fold greater than those of methoxide-promoted reactions. The synergetic effect in the cleavage of HPNPP with Zn_2-**8** is 561-fold. In the presence of 1 mM of Zn_2-**8** at self-buffered conditions, the methanolysis of HPNPP and MPNPP can be accelerated by 4.4 × 10^{10}-fold and 8.4 × 10^9-fold, respectively.

Conclusions

The present work has systematically investigated the cleavage of RNA and DNA model phosphates HPNPP and MPNPP catalyzed by zinc(II) complexes of the *N*-methylated mono- and

di-[12]aneN$_3$ ligands (**5**–**9**). The obtained results revealed that *N*-methylation of the [12]aneN$_3$ backbone greatly reduced the cooperative behavior among the mononuclear Zn(II) complexes, a common phenomenon in a reduced dielectric alcohol medium. Such cooperative action would result in the synergetic effect and significant catalytic activity. The activities of the dinuclear zinc(II) complexes for the di-[12]aneN$_3$ ligands containing di-*N*-methyl (**8**) and tetra-*N*-methyl (**9**) substituents for cleaving HPNPP have been impaired by 91-fold and 63 000-fold, respectively. The reduction of the activity in the cleavage of MPNPP follows a similar trend. The kinetic results indicate that the steric effects derived directly from the coordinating nitrogen donors greatly reduced the binding activation process and accumulated exponentially. Nevertheless, some zinc(II) complexes of *N*-methylated mono- and di-[12]aneN$_3$ ligands still provide significant catalytic efficiency in methanol. Dinuclear complex Zn_2 : **8** can accelerate the cleavage of each phosphate by a factor of 10^{9-10} folds relative to the background reactions at concentration of 1 mM and self-buffered conditions. The synergetic effects between the two [12]aneN$_3$ units of Zn_2-**8** are 561 and 340 folds, respectively, for the catalytic cleavage of the two phosphates. Results from this work have provided the insight into the steric effects on reactions catalyzed by artificial nucleases and further confirmed that a combination of the cooperative effect between two metal ions and a solvent effect can lead to significant catalytic activities for the cleavage of phosphate diesters.

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27 For the designation of pHin non-aqueous solvents we use the forms recommended by the IUPAC, *Compendium of Analytical Nomenclature. Definitive Rules 1997* 3rd edn, Blackwell, Oxford, U.K. 1998. If one calibrates the measuring electrode with aqueous buffers and then measures the pH of an aqueous buffer solution, the term $^{w}_{w}$pH is used; if the electrode is calibrated in water and the 'pH' of the neat buffered methanol solution then measured, the term $^{s}_{w}$pH is used; and if the electrode is calibrated in the same solvent and the 'pH' reading is made, then the term $^{s}_{s}$pH is used. Since the autoprotolysis constant of methanol is $10^{-16.77}$, neutral $^{s}_{s}$pH is 8.4
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