# Mono-Schiff base complexes with aza-crown ether or morpholino pendants as synthetic hydrolases for *p*-nitrophenyl picolinate cleavage

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Received: 19 August 2012/Accepted: 6 November 2012/Published online: 29 November 2012 © Springer Science+Business Media Dordrecht 2012

Abstract Mono-Schiff base complexes of Co(II) and Mn(III) with either benzo-10-aza-crown ether pendants  $(MnL_2^1Cl, MnL_2^2Cl)$  or morpholino pendants  $(MnL_2^3Cl,$  $CoL_2^3$ ) have been employed as models for hydrolase enzymes by studying the kinetics of their hydrolysis reactions with p-nitrophenyl picolinate (PNPP). A kinetic model of PNPP cleavage catalyzed by these complexes is proposed. The effects of complex structures and reaction temperature on the rate of catalytic PNPP hydrolysis have also been examined. The rate increases with pH of the buffer solution; all four complexes exhibited high activity in the catalytic PNPP hydrolysis. Compared with the crown-free analogs  $MnL_2^3Cl$ and CoL<sub>2</sub><sup>3</sup>, the crowned Schiff base complexes (MnL<sub>2</sub><sup>1</sup>Cl, MnL<sub>2</sub><sup>2</sup>Cl) exhibited higher catalytic activity. The pseudo-firstorder rate constant for the PNPP hydrolysis catalyzed by the complex  $MnL_2^1Cl$ , containing a benzo-10-aza-crown ether pendant, is  $1.84 \times 10^3$ -fold higher than that of spontaneous hydrolysis of PNPP at pH 7.60, 25 °C,  $[S] = 2.0 \times 10^{-4}$ mol  $dm^{-3}$ .

# Introduction

Enzyme mimics are artificial compounds whose structures are based on our current understanding of enzymes and

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their specific attributes for hydrolysis of biological molecules. Studies of synthetic molecules as hydrolase mimetics have received considerable attention [1]. Recently, there has been much effort to design and synthesize model complexes for promoting the hydrolysis of carboxylate and phosphate esters, due to important environmental and biological applications [2, 3]. In recent years, various chemical systems have been used as models for natural enzymes [4-9]. These artificial hydrolases have similar catalytic function to natural enzymes owing to their active groups and structures which are similar to the natural enzymes; moreover, they are structurally more simple and stable than natural enzymes and can provide information on the mechanistic aspects of enzyme action. Our research group has focused on such enzyme mimics, especially transition metal complexes with crowned Salen-type or unsymmetrical bis-Schiff base ligands as catalysts for the hydrolysis of esters in buffered solution [10–14]. The crown ether-containing Schiff bases have attracted much attention because the crown rings could endow functional molecules with novel performance and character, owing to the hydrophobicity of the outer ethylene groups and orderly arrangement of inner oxygen atoms [15].

Previously, the catalytic performance of mono-Schiff base complexes with 3-substituted aza-crown ether or morpholino pendants in *p*-nitrophenyl picolinate (PNPP) hydrolysis was reported [16, 17]. With hopes of further investigating the reaction kinetics and mechanism of PNPP hydrolysis catalyzed by mono-Schiff base complexes with 5-substituted aza-crown ether or morpholino pendants, mono-Schiff base Co(II) and Mn(III) complexes with benzo-10-aza-crown ether pendants (MnL $_2^1$ Cl, MnL $_2^2$ Cl), and the analogs with morpholino pendants (MnL $_2^1$ Cl, CoL $_2^3$ ) have been employed as models to mimic enzymatic hydrolysis of PNPP. In this paper, we have investigated the

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 $R=NO_2$ ,  $MnL_2^1Cl$ ; R=Cl,  $MnL_2^2Cl$ 

Fig. 1 Structures of mono-Schiff base complexes

effects of the position of substituents and the kind of metal on the catalytic performance of the Schiff base complexes with aza-crown ether or morpholino pendants for PNPP hydrolysis. The structures of these mono-Schiff base complexes are shown in Fig. 1.

## Experimental

### Materials and methods

Kinetic and thermodynamic measurements were taken by UV-vis methods with a GBC 916 UV-vis spectrophotometer (GBC Co., Australia) equipped with a thermostatic cell holder. All reagents, unless otherwise indicated, were of analytical grade and used without further purification. Trihydroxymethylaminomethane (Tris) was purchased from Aldrich. Buffer solutions were made from standardized nitric acid. Water was obtained on a Water Purification System (Nex Power 1000, Human Corporation, South Korea) to achieve a resistivity of at least 16 M $\Omega$  cm<sup>-1</sup>. The ionic strength of the buffer solution was maintained at  $0.1 \text{ mol } \text{dm}^{-3} \text{ KNO}_3$  throughout the experiments. The pH value of the buffer solution was measured at 25 °C using a Radiometer PHM 26 pH meter (made in China) fitted with G202C glass and K4122 calomel electrodes. The following compounds were prepared according to the literature: PNPP [18], and the four mono-Schiff base Co(II) and Mn(III) complexes (MnL $_2^1$ Cl, MnL $_2^2$ Cl, MnL $_2^3$ Cl, CoL $_2^3$ ) [19, 20]. PNPP stock solution for kinetics was prepared in acetonitrile.

# Kinetics studies

The pseudo-first-order rate constants  $(k_{obs})$  were obtained based on the initial rate method, that is, according to the equations:

$$(\text{rate})_0 = -(dC/dt)_0 = (dA/dt)_0/\epsilon$$
$$(\text{rate})_0 = k_{\text{obs}}[\text{ML}]_0,$$

Plots of  $(rate)_0$  versus  $[ML]_0$  were used to obtain the pseudo-first-order rate constants from the slope of the

M=MnCl, MnL<sup>3</sup><sub>2</sub>Cl; M=Co, CoL<sup>3</sup><sub>2</sub>

straight lines, where  $(rate)_0$  is the initial rate of PNPP hydrolysis and  $[ML]_0$  is the initial concentration of the complex.

Each kinetic run was initiated by injecting an acetonitrile solution of concentration PNPP at the desired concentration into a 1-cm cuvette containing 3 cm<sup>3</sup> of the complex at the desired concentration. The pseudo-first-order rate constants for PNPP hydrolysis were determined by monitoring the release of *p*-nitrophenol at 400 nm under the conditions of more than 20-fold excess of substrate over catalyst. The molar extinction coefficients ( $\varepsilon$ , unit as L mol<sup>-1</sup> cm<sup>-1</sup>) for *p*-nitrophenol at 400 nm were obtained by measuring the absorption of desired concentrations of *p*-nitrophenol at various pH values and listed as follows: pH 7.60,  $1.37 \times 10^4$ ; pH 7.90,  $1.58 \times 10^4$ ; pH 8.20,  $1.72 \times 10^4$ ; pH 8.50,  $1.81 \times 10^4$ ; pH 8.80,  $1.86 \times 10^4$ .

### **Results and discussion**

Rate constants for PNPP hydrolysis

The pseudo-first-order rate constants obtained for catalytic PNPP hydrolysis by these Schiff base complexes are shown in Table 1. The pseudo-first-order rate constant ( $k_0$ ) for PNPP hydrolysis in the absence of catalyst is  $1.95 \times 10^{-5} \text{ s}^{-1}$  at pH = 7.60, 25 °C, [S] =  $2.0 \times 10^{-4}$  mol dm<sup>-3</sup>. Hence, the rate of the PNPP hydrolysis catalyzed by the complexes increases by a factor of ca.  $1.84 \times 10^3$  times for MnL<sub>2</sub><sup>1</sup>Cl,  $1.74 \times 10^3$  times for MnL<sub>2</sub><sup>2</sup>Cl,  $1.40 \times 10^3$  times for MnL<sub>2</sub><sup>2</sup>Cl, and  $1.32 \times 10^3$  times for CoL<sub>2</sub><sup>3</sup>, and the catalytic activity follows the order MnL<sub>2</sub><sup>1</sup>Cl > MnL<sub>2</sub><sup>2</sup>Cl > MnL<sub>2</sub><sup>3</sup>Cl > CoL<sub>2</sub><sup>3</sup> under the same experimental conditions.

In contrast, the values of  $k_{obs}$  for PNPP hydrolysis catalyzed by the free Schiff bases HL<sup>1</sup>, HL<sup>2</sup>, and HL<sup>3</sup> in buffered solution are  $5.93 \times 10^{-4}$ ,  $5.26 \times 10^{-4}$ , and  $4.97 \times 10^{-4} \text{ s}^{-1}$ , respectively, and the  $k_{obs}$  values for the hydrolysis catalyzed by the simple metal salts in buffered solution are  $8.16 \times 10^{-4} \text{ s}^{-1}$  for CoCl<sub>2</sub> and  $8.13 \times 10^{-4} \text{ s}^{-1}$  for MnCl<sub>2</sub> under the same conditions. Hence, these Schiff base complexes are much better catalysts than the free Schiff base ligands or simple metal salts.

### Kinetic model of PNPP hydrolysis

The catalytic hydrolysis of PNPP by these complexes can be expressed as in Scheme 1 involving Eqs. (1) and (2), where S is the substrate PNPP, ML is the hydrated complex, MLS represents the intermediate adduct of ML and PNPP, P represents the product *p*-nitrophenol, *K* is the association constant between the PNPP and ML, and *k* is the first-order rate constant for the product formation, which is pH-dependent. The constant  $k_0$  is the pseudo-first-order rate constant for PNPP hydrolysis in the absence of catalyst.

**Table 1** Pseudo-first-order rate constants  $(k_{obs})$  of catalytic PNPP hydrolysis in buffer solutions

Complex	10 <sup>4</sup> [PNPP]/ (mol dm <sup>-3</sup> )	$10^2 k_{\rm ob}$	$10^2 k_{\rm obs} / {\rm s}^{-1}$					
		рН 7.60	рН 7.90	рН 8.20	рН 8.50	рН 8.80		
MnL <sup>1</sup> <sub>2</sub> Cl	2.00	3.58	8.74	23.42	38.67	52.29		
	2.67	4.68	11.81	28.24	51.96	68.53		
	3.33	5.86	14.19	35.11	60.58	82.21		
	4.00	6.97	16.45	41.95	68.11	90.32		
	4.67	7.96	19.09	49.82	76.92	99.78		
MnL <sub>2</sub> <sup>2</sup> Cl	2.00	3.40	7.99	21.87	36.96	48.98		
	2.67	4.32	11.12	27.17	45.96	62.59		
	3.33	5.51	13.24	32.35	54.58	75.21		
	4.00	6.55	15.84	37.13	63.45	87.38		
	4.67	7.46	16.41	46.54	73.92	89.18		
MnL <sub>2</sub> <sup>3</sup> Cl	2.00	2.73	7.14	20.14	36.96	44.94		
	2.67	3.43	9.42	23.84	44.34	57.22		
	3.33	4.36	10.89	28.71	51.58	65.21		
	4.00	5.15	13.12	34.79	58.45	73.38		
	4.67	5.97	15.14	38.41	69.92	82.18		
CoL <sub>2</sub> <sup>3</sup>	2.00	2.58	6.76	18.44	36.26	43.99		
	2.67	3.31	9.17	22.24	43.14	54.72		
	3.33	3.96	9.97	26.56	49.58	62.91		
	4.00	4.96	12.72	32.21	56.05	71.18		
	4.67	5.61	14.11	34.19	66.98	76.18		

Conditions: (25  $\pm$  0.1) °C, I = 0.1 mol dm  $^{-3}$  KNO3, [complex] = 1.0  $\times$  10  $^{-5}$  mol dm  $^{-3}$ 



Since the rate of PNPP spontaneous hydrolysis is much lower than that of catalytic hydrolysis, the products of spontaneous hydrolysis of PNPP can be neglected in the kinetic calculations. Hence, Scheme 1 leads to the rate equation:

$$Rate = k[MLS]$$
(3)

The association constants K can be expressed in terms of concentrations:

$$K = [MLS]/[ML][S]$$
(4)

According to the material balance, we have:

$$[ML]_{T} = [ML] + [MLS]$$
(5)

Combination of Eqs. (4) and (5) leads to:

$$[MLS] = \frac{K[S][ML]_{T}}{1 + K[S]}$$
(6)

Combination of Eqs. (6) and (3) followed by rearrangement gives:

$$rate = \frac{kK[S][ML]_{T}}{1 + K[S]} = k_{obs}[ML]_{T}$$
(7)

$$\frac{1}{k_{obs}} = \frac{1}{k} + \frac{1}{Kk[\mathbf{S}]} \tag{8}$$

In the above equations, [ML] and [ML]<sub>T</sub> are the free and total concentration of the hydrated complex, respectively; [S] is the concentration of free substrate and can be substituted by the initial concentration of the substrate based on the initial rate method; [MLS] is the concentration of the intermediate formed by the substrate and the hydrated complex; and  $k_{obs}$  is the pseudo-first-order rate constant.

Based on the experimental data (see Table 1) and Eq. (8), the relationships between  $1/k_{obs}$  versus 1/[S] were plotted in Fig. 2, showing a good linear relationship between the variables, with  $r^2 > 0.98$ . The plots have a positive intercept that evaluates the first-order rate constant k with relative standard deviation of less than 1 %. The calculated values of k are listed in Table 2.







Fig. 2 Effect of substrate concentration on first-order rate constants for catalytic PNPP hydrolysis by the complexes  $MnL_2^1Cl$ ,  $MnL_2^2Cl$ ,  $MnL_2^3Cl$ , and  $CoL_2^3$  at 25 °C, I = 0.1 mol.dm<sup>-3</sup> KNO<sub>3</sub>, [Tris] = 0.1 mol dm<sup>-3</sup>,

**Table 2** k (s<sup>-1</sup>) of the catalytic hydrolysis of PNPP by mono-Schiff base complexes in buffer solutions at various pH

pН	pH 7.60	pH 7.90	pH 8.20	pH 8.50	pH 8.80
$k(MnL_2^1Cl)$	1.155	1.582	2.299	2.973	3.378
$k(MnL_2^2Cl)$	0.842	1.313	1.784	2.527	2.901
$k(MnL_2^3Cl)$	0.525	0.793	1.176	1.686	2.073
k (CoL <sub>2</sub> <sup>3</sup> )	0.439	0.685	1.028	1.474	1.754

# $[\text{complex}] = 1.0 \times 10^{-5} \text{ mol dm}^{-3}$ , filled diamond pH 7.60, filled square pH 7.90, filled triangle pH 8.20, filled circle pH 8.50, asterisk pH 8.80



Scheme 2 The process of proton transfer at the rate-determining step

### pH profile and stoichiometry

Hydrolases are found to be very sensitive to the acidity of the physiological microenvironment. The spatial conformation of the enzyme may be transformed by the changes in the pH, and the stability of the intermediate (MLS) should also be changed. From the results obtained in the present study, it is clear that the first-order rate constant (*k*) is pH-dependent. It is most likely that *k* is related to the acid dissociation constant  $K_a$  of H<sub>2</sub>O coordinated to the metal center. The value of *k* increased with increasing pH from 7.60 to 8.80. This implies that the reaction process contains a proton transfer at the rate-determining step in Scheme 1, which can be shown as in Scheme 2.

 $K_a$  is the acid dissociation constant of H<sub>2</sub>O coordinated to the metal center, and  $k_1$  is the first-order rate constant, which is pH-independent. In this process, the intermediate MLS was first ionized and then internal electron transfer within the intermediate MLS formed the products. The rate for PNPP catalytic hydrolysis depends on the stabilization of the intermediate MLS<sup>-</sup>. According to the principles of chemical equilibration, it is more favorable for the intermediate MLS to form the intermediate  $MLS^-$  and generate the products in alkaline solution. Hence, the first-order rate constants (*k*) increase with the increase in pH value in the reaction system.

This leads to the following analysis:

$$K_{\rm a} = [\rm H^+][\rm MLS^-]/[\rm MLS] \tag{9}$$

According to the material balance, we have:

$$[MLS] = [MLS]_t + [MLS^-]$$
(10)

Combination of Eqs. (9) and (10) leads to:

$$[\mathrm{MLS}^{-}] = \frac{K_{\mathrm{a}}[\mathrm{MLS}]}{[\mathrm{H}^{+}] + K_{\mathrm{a}}}$$
(11)

The rate equation in Scheme 2 can then be expressed as:

$$k[\mathrm{MLS}] = k_1[\mathrm{MLS}^-] \tag{12}$$

Combination of Eqs. (12) and (13) and rearrangement leads to:

$$\frac{1}{k} = \frac{1}{k_1} + \frac{1}{k_1 K_a} [\mathrm{H}^+]$$
(13)

where [MLS<sup>-</sup>] is the dissociated concentration of the intermediate (MLS), and [MLS]<sub>t</sub> is the undissociated concentration of the intermediate (MLS). On the basis of Eq. (13), the  $k_1$  and  $K_a$  values can be obtained from the slope and intercept of the plot 1/k versus [H<sup>+</sup>] (Fig. 3). This gives  $k_1$  and  $pK_a$  values of 3.64 s<sup>-1</sup> and 7.95 for MnL<sub>2</sub><sup>1</sup>Cl, 3.30 s<sup>-1</sup> and 8.07 for MnL<sub>2</sub><sup>2</sup>Cl, 2.32 s<sup>-1</sup> and 8.14 for MnL<sub>2</sub><sup>3</sup>Cl, and 2.07 s<sup>-1</sup> and 8.18 for CoL<sub>2</sub><sup>1</sup>, respectively.



**Fig. 3** pH rate profile for the catalytic hydrolysis of PNPP by the complexes in the buffer solution at 25 °C (*filled diamond* MnL<sub>2</sub><sup>1</sup>Cl, *filled square* MnL<sub>2</sub><sup>2</sup>Cl, *filled triangle* MnL<sub>2</sub><sup>3</sup>Cl, *filled circle* CoL<sub>2</sub><sup>3</sup>)

Effects of the complex structures

Factors affecting the activity of Schiff base complexes as enzyme mimics in ester hydrolysis include steric hindrance, and electronic effects of the substituents on the Schiff base ligand provide a hydrophobic microenvironment for PNPP close to the catalytic site. The hydrolase mimics reported in this paper exhibit the similar structural effects to those of the natural hydrolase in PNPP catalytic hydrolysis. Tables 1 and 2 show that the catalytic activities of the crowned Schiff complexes are higher than those of crown-free analogs  $(MnL_2^2Cl > MnL_2^3Cl)$ . This can be attributed to the crown ether substituent in the Schiff base complexes. Its hydrophobic environment, provided by its outer ethylene groups, means that the hydrophobic PNPP molecule interacts more favorably with these complexes; on the other hand, as hydrogen bonds may be formed between the oxygen atoms of the crown ether and H<sub>2</sub>O in the intermediate MLS, H<sub>2</sub>O coordinated to the metal can be activated synergistically by both the metal and crown; hence, the formation of the intermediate MLS<sup>-</sup> may be faster in the crown Schiff complexes. This result is, however, opposite to that published previously [21]. We think that the differences in geometric factors and steric hindrance between benzoaza-15-crown-5 and morpholine may be definitive factors when these substituents occupy position 3, whereas the difference in hydrophobicities between these two substituents may be the definitive factor when they occupy position 5. The steric hindrance of the 5- substituents for the approach of PNPP is smaller than that of the 3-substituted analogs. However, the hydrophobic microenvironment provided by benzoaza-15crown-5 when at position 5 may be the dominant factor for PNPP, rather than steric hindrance. It can also be seen that the catalytic activity of the Mn(III) complex is higher than that of the corresponding Co(II) complex with the same ligands ( $MnL_2^3Cl > CoL_2^3$ ). This can be explained by the higher charge density of Mn(III) compared to Co(II), resulting from the smaller radius and higher positive charge of Mn(III). The smaller the metal radius, the stronger the ability of activating H<sub>2</sub>O and binding PNPP, increasing rate of PNPP hydrolysis; on the other hand, the higher the positive charge of central metal, the faster the proton transfer of coordinated H<sub>2</sub>O to give coordinated hydroxide OH<sup>-</sup>. In this way, coordinated water becomes a good nucleophile in the form of hydroxide around neutral pH for the attack of PNPP, as observed in metallohydrolases [22]. The obtained  $k_{obs}$  and k values listed in Tables 1 and 2 show that  $MnL_2^1Cl$  possesses a higher activity than  $MnL_2^2Cl$ . This could be due to the positive charge density of the metal being enhanced by an electron-withdrawing effect of the substituent on the amino aromatic ring. Since the electronwithdrawing effect of the substituents follows the order  $NO_2 > Cl$ , the positive charge on Mn(III) in MnL<sub>2</sub><sup>1</sup>Cl will be higher than that in MnL<sub>2</sub><sup>2</sup>Cl; hence, MnL<sub>2</sub><sup>1</sup>Cl readily accelerates the deprotonation of the water ligand, which can be confirmed by the smaller  $pK_a$  value (7.95) of MnL<sub>2</sub><sup>1</sup>Cl relative to that (8.07) of MnL<sub>2</sub><sup>2</sup>Cl. Compared with catalytic activity of mono-Schiff base complexes bearing an electron-donating methyl group, as reported in the literature [23], the catalytic activities of these mono-Schiff base complexes bearing electron-withdrawing substituent groups (NO<sub>2</sub> or Cl) are larger under comparable conditions.



**Fig. 4** The plot of -ln  $k_{obs}$  versus  $T^{-1}$  at pH = 7.60, [S] = 3.33 × 10<sup>-4</sup> mol dm<sup>-3</sup>, [complex] = 1.0 × 10<sup>-5</sup> mol dm<sup>-3</sup>. (filled diamond MnL<sub>2</sub><sup>1</sup>Cl, filled square MnL<sub>2</sub><sup>2</sup>Cl, filled triangle MnL<sub>2</sub><sup>3</sup>Cl, asterisk CoL<sub>2</sub><sup>3</sup>)

### Effect of temperature

Temperature is another important factor that influences enzyme efficiency. However, an enzyme may become denatured and lose all functions at higher temperatures. In order to investigate the effects of temperature on the present system, the pseudo-first-order rate constants  $(k_{obs})$  of PNPP hydrolysis were obtained at five different temperatures from 25 to 65 °C at pH = 7.60, [S] =  $3.33 \times 10^{-4}$  mol dm<sup>-3</sup>. According to the Arrhenius equation, straight lines were obtained from plots of  $-\ln k_{obs}$  versus 1/T (Fig. 4). The apparent activation energies  $(E_a)$  were determined from the slopes of the straight lines, giving  $E_a$  values of  $16.01 \text{ kJ mol}^{-1}$  for MnL<sub>2</sub><sup>1</sup>Cl,  $16.34 \text{ kJ mol}^{-1}$  for MnL<sub>2</sub><sup>2</sup>Cl, 17.50 kJ mol<sup>-1</sup> for MnL<sub>2</sub><sup>3</sup>Cl, and 18.23 kJ mol<sup>-1</sup> for CoL<sub>2</sub><sup>3</sup>. This further indicates that all four complexes are stable in the temperature range investigated. Since the formation rate of the intermediates, MLS and MLS<sup>-</sup>, should increase with increasing temperature, the rate of PNPP catalytic hydrolysis is enhanced.

### Proposed mechanism for catalytic hydrolysis

The proposed mechanism of the catalytic hydrolysis of PNPP by  $MnL_2^1Cl$ , which is used as an example, is outlined in Scheme 3 on the basis of previous reports [24–26] and the  $pK_a$  values of the complexes noted above. Coordination of H<sub>2</sub>O to the Mn(III) atom would result in a hydrated complex in aqueous solution, which may be the active



Scheme 3 Proposed mechanism for the hydrolysis of PNPP catalyzed by mono-Schiff base complexes with benzo-10-aza-crown ether pendant

species for the PNPP catalytic hydrolysis [27]. Therefore, we assume that the mechanism of PNPP catalytic hydrolysis is similar to that of analogous hydrolytic metalloenzyme.

The coordinated H<sub>2</sub>O is then synergistically activated by the metal and crown ring, forming an intramolecular hydrated complex (ML). Coordination of PNPP then gives the intermediate MLS (step I). Next, the coordinated hydroxide attacks the carbonyl group of PNPP with a firstorder rate constant (k) (step II), which is the overall ratedetermining step. Finally, H<sub>2</sub>O rapidly re-binds and picolinic acid is released from the metal (step III). According to previous reports [8, 28], the catalytic hydrolysis of PNPP may involve free OH<sup>-</sup> acting as a nucleophile when the pseudo-first-order rate constant for PNPP hydrolysis is fairly independent of substrate concentration. However, from Fig. 2, it can be seen that the pseudo-first-order rate constants for PNPP hydrolysis increase linearly with substrate concentration at any pH, indicating that the hydrated complex (MLS<sup>-</sup>) may be the real active species for PNPP catalytic hydrolysis.

### Conclusion

In this report, we have investigated the catalytic hydrolysis of PNPP by mono-Schiff base Mn(III) and Co(II) complexes with benzo-10-aza-crown ether or morpholino pendants. The rate of the catalytic PNPP hydrolysis was increased with increasing pH of the buffer solution. The catalytic activity of the complex with 5-substituted benzoaza-15-crown-5 is higher than that of the 5-substituted morpholine analog, while the catalytic activity of mono-Schiff base complexes bearing an electron-withdrawing substituent (NO<sub>2</sub> or Cl) on the amino aromatic ring is larger than that of the analogous mono-Schiff base complex bearing an electron-donating methyl group. The pseudofirst-order rate for PNPP hydrolysis catalyzed by the complex MnL<sub>2</sub><sup>1</sup>Cl is  $1.84 \times 10^3$  times than that of spontaneous hydrolysis of PNPP. These mono-Schiff base complexes are stable in the range of temperature of 25-65 °C in this work.

Acknowledgments The authors gratefully acknowledge financial support from China National Natural Science Foundation (No.20072025), Key Scientific and Technological Project Issued by Ministry of Education of China (No. 208118), and the Opening Project of Key Laboratory of Green Catalysis of Sichuan Institutes of High Education (No. LZJ01, LZJ1101).

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