Effect of cyclodextrin dimers with bipyridyl and biphenyl linking groups on carboxyl ester hydrolysis catalyzed by zinc complex

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ABSTRACT

Two new β-cyclodextrin dimers, biphenyl-4,4′-bis(6-monodeoxy-6-ammoniomethyl-β-cyclodextrin) (Host 1, **H1**) and 2,2′-bipyridyl-5,5′-bis(6-monodeoxy-6-ammoniomethyl-β-cyclodextrin) (Host 2, **H2**), were synthesized and further assembled with a zinc complex containing a hydrophobic group to construct two host–guest systems, ZnL(**H1**) and ZnL(**H2**) (L = 4-(4′-*tert*-butylbenzyl)diethylenetriamine), acting as supramolecular metallohydrolase models. In such system, the chemical equilibrium constants were determined by pH potentiometric titration at 298 ± 0.1 K. Two deprotonation constants of the Zn(II)-coordinated water, 8.68 ± 0.03 (pK_{a1}) and 10.50 ± 0.04 (pK_{a2}), were obtained respectively for the ZnL(**H1**) system whereas only one pK_{a1} value was obtained for ZnL(**H2**) (9.38 ± 0.02). The kinetics of *p*-nitrophenyl acetate (pNA) hydrolysis catalyzed by ZnL(**H1**) and ZnL(**H2**) were examined respectively from pH 7.56 to 10.56 at 298 ± 0.1 K. The pH profile of rate constant of pNA hydrolysis catalyzed by ZnL(**H1**) exhibits an exponential increase with second-order rate constants of 0.98 and 8.41 $M^{-1}\,s^{-1}$ for mono- and di-hydroxyl active species, indicating a potent catalytic activity relative to the reported mononuclear and polynuclear Zn(II) species. However, the pH profile of rate constant of hydrolysis catalyzed by ZnL(**H2**) only shows a saturated kinetic behavior with the second-order rate constant of 1.26 $M^{-1}\,s^{-1}$, suggesting a kinetic process controlled only by an acid–base equilibrium. All kinetic results are in good agreement with thermodynamic data for hydroxyl activated species in ZnL(**H1**) and ZnL(**H2**) systems.

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1. Introduction

Artificial metalloenzymes are enzyme-like hybrid catalysts resulting from the introduction of a catalyst into macromolecular hosts, which provide a well-defined second coordination sphere by immobilizing the substrate around the active site with multiple recognition and thus induce the chemo-, region-selectivity of the reaction [1,2]. The chiral hydrophobic pocket provided by hosts is expected to supramolecular anchoring for substrate or guest to make catalyst–substrate complex pre-organized for the reactive geometry [3,4]. Cyclodextrins (CDs) hosts are able to encapsulate various inorganic/organic guest molecules into hydrophobic cavities and have been extensively studied in the fields of artificial enzymes and molecular devices [5,6]. Linking two β-CD hydrophobic cavities in close vicinity by an appropriate bridge group, Bis(cyclodextrin)s (BisCDs) behave intramolecular cooperative binding and multiple weak interactions towards guest molecules in aqueous solution [7]. Breslow pioneered in the

biomimetic work of introducing the cyclodextrin hydrophobic cavity to guide selectivity for substrate hydrolysis catalyzed by metal complex [8]. Since then, to mimic the substrate-specific interaction of metalloenzyme, a variety of BisCDs with different functional linking groups have been documented, such as telluroxy, phenanthroline, porphyrins and tris(2-aminoethyl)amine [9–12]. However, metalloenzyme mimics constructed by host–guest system of cyclodextrin inclusion complex, in which a metal complex bind a CD molecule, are still rarely reported. Recently, we reported some cyclodextrin inclusion complexes as enzyme mimics [13–15], in which the metal complex was assembled into the hydrophobic cavity of CD to serve as a supramolecular metalloenzyme model. Indeed, some properties of zinc complex can be improved by the introduction of a CD, such as lowering the pK_a values of Zn^{2+}-coordinated water molecules, preventing the formation of the hydroxyl-bridged dimer of zinc complex and stabilizing hydroxyl species in solution. But for the supramolecular system of the CD dimer linked by the 4,4′-dimethyl-2,2′-bipyridyl group, the catalytic activity for ester hydrolysis still awaits to be improved. Since a subtle change in the substitution pattern of the bipyridine unit have a marked effect on the binding affinities toward the guest for the CD dimer tethered from the secondary side [16], we subsequently

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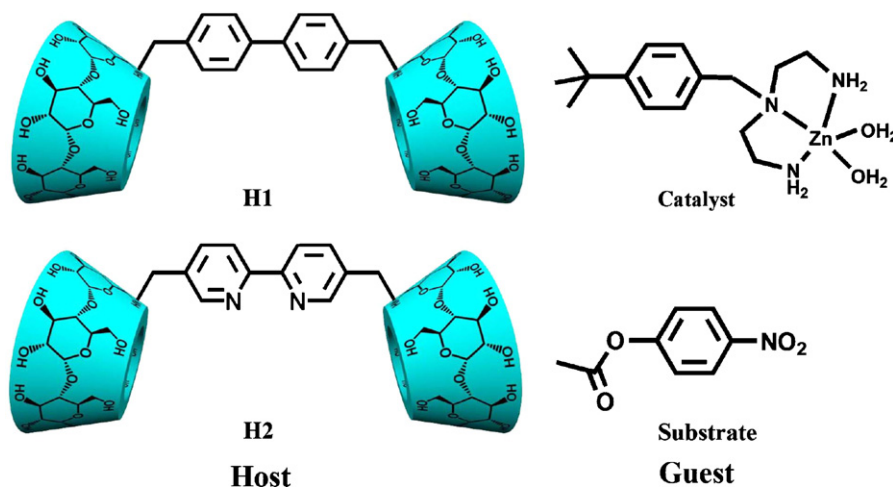
Scheme 1. Host (**H1** and **H2**), catalyst (ZnL) and substrate (pNA).

design and synthesize two new CD dimers bridged from the primary side with 5,5′-dimethyl-2,2′-bipyridyl and 4,4′-dimethyl-biphenyl. One of the two CD cavities is assembled with the catalyst of a Zn(II) complex, forming a supramolecular model for a metallohydrolase. The other cavity will be applied to fix a substrate in catalytic process. Herein, we report their synthesis and characterization, thermodynamic properties, and catalytic activities of *p*-nitrophenyl acetate (pNA) hydrolysis.

2. Experimental

2.1. Materials

Biphenyl-4,4′-dicarbaldehyde, 5,5′-dimethyl-2,2′-bipyridyl and *p*-nitrophenyl acetate were purchased from Aldrich Chemical Company. β-CD of reagent grade was recrystallized twice from H$_2$O and dried in vacuo for 12 h at 373 K, DMF were dried over CaH$_2$ for 2 days and then distilled under reduced pressure prior to use. Common organic reagents were reagent grade and redistilled before use. Water used in all physical measurement experiments was Milli-Q grade. L (L=4-(4′-tertbutylbenzyl)diethylenetriamine), ZnL (Scheme 1) was prepared

as our previous work [14,15]; 6-monodeoxy-6-monoamino-β-cyclodextrin was synthesized according to previous procedure [17,18]; 5,5′-dibromomethyl-2,2′-bipyridyl was obtained according to the literature procedure [19]. Biphenyl-4,4′-bis(6-monodeoxy-6-ammoniomethyl-β-cyclodextrin) (**H1**) and 2,2′-bipyridyl-5,5′-bis(6-monodeoxy-6-ammoniomethyl-β-cyclodextrin) (**H2**) was prepared as Scheme 2. All compounds were confirmed by their elemental analyses, ESI-MS, IR and ^{1}H NMR spectra (Figs. S1–S3).

2.1.1. Synthesis of biphenyl-4,4′-bis(6-monodeoxy-6-ammoniomethyl-β-cyclodextrin), H1

To a solution of 6-monodeoxy-6-monoamino-β-cyclodextrin (2.395 g, 2.112 mmol) in the mixture solvent of dry DMF and anhydrous MeOH (2:1, v/v) was dropwisely added a solution of biphenyl-4,4′-dicarbaldehyde (0.249 g, 1.056 mmol) in 5 mL dry DMF with vigorous stirring. After heated to 353 K for 6 h under nitrogen, the reaction was cooled to room temperature. Then slight excessive NaBH$_4$ (0.096 g, 2.543 mmol) was added to reduce imine for 2 h, followed by water wash and filtration, and then filtrate was evaporated under a reduced pressure to dryness. The residue was dissolved in a small amount of hot water, and the aqueous solution was poured into acetone (200 mL) to give a white precipitate.
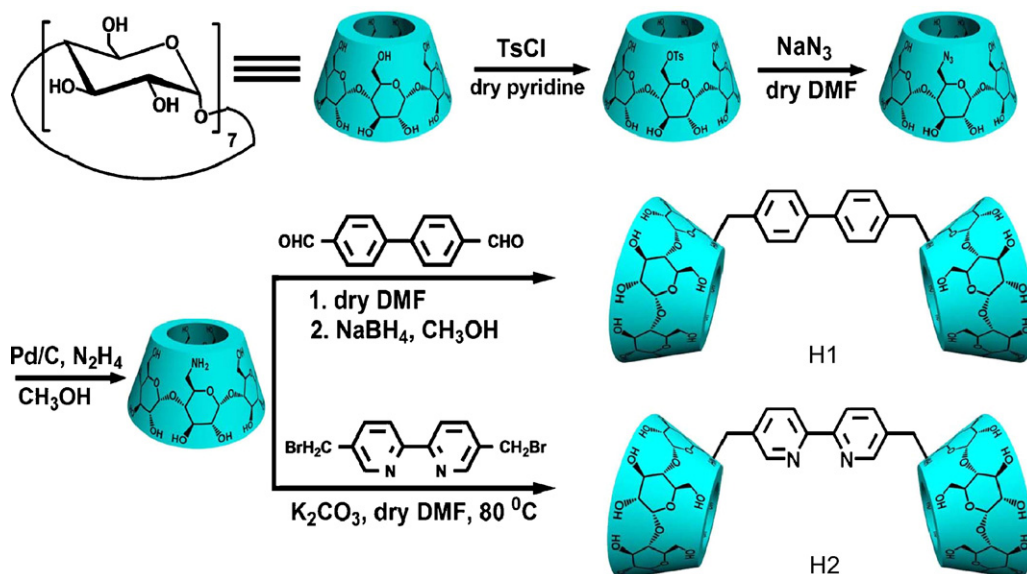
Scheme 2. Synthetic routes of **H1** and **H2**.

The crude product obtained was purified by column chromatography over Sephadex G-25 with distilled deionized water as an eluent to give pure compound in 53% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.76 (d, ^3J(H,H) = 8.2 Hz, 4H; biphenyl-H-2,2′), 7.66 (d, ^3J(H,H) = 8.2 Hz, 4H; biphenyl-H-3,3′), 5.68–5.93 (m, 28H, OH-2,3), 4.72–4.96 (m, 14H, H-1), 4.50 (m, 12H, OH-6), 3.91–3.46 (m, 60H, H-3,5,6, biphenyl-CH$_2$), 3.36–3.17 (m, 28H, H-2,4; overlaps with H$_2$O), 2.08 (brs, 2H, NH); IR (cm^{-1}, KBr) 3379, 2929, 2106, 1641, 1415, 1369, 1157, 1081, 1027, 945, 846, 816, 755, 707, 578, 529; MS (ESI, H$_2$O/MeOH, 1:1, v/v, m/z): [M+2H]$^{2+}$ calcd 1223.9, found 1223.7, [M+Na+H]$^{2+}$ calcd 1234.6, found 1234.5; Anal. calcd for C$_{98}$H$_{152}$N$_2$O$_{68}$·18H$_2$O: C 42.49, H 6.84, N 1.01. Found: C 42.32, H 6.74, N, 1.03.

2.1.2. Synthesis of 2,2′-bipyridyl-5,5′-bis(6-monodeoxy-6-ammoniomethyl-β-cyclodextrin), H2

To a solution of 6-monodeoxy-6-monoamino-β-cyclodextrin (2.395 g, 2.112 mmol) and anhydrous potassium carbonate (1.166 g, 8.448 mmol) in dry DMF was dropwisely added a solution of 5,5′-dibromomethyl-2,2′-bipyridyl (0.359 g, 1.056 mmol) in 5 mL dry DMF with vigorous stirring. After heated to 353 K for 8 h under nitrogen, the reaction was cooled to room temperature, and then filtrated and evaporated to dryness under a reduced pressure. The residue was dissolved in a small amount of hot water, and the aqueous solution was poured into acetone (200 mL) to give a white precipitate. The crude product obtained was purified by column chromatography over Sephadex G-25 with distilled deionized water as an eluent to give pure compound in 41% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.57 (s, 2H; bipyridyl-H-6,6′), δ 8.30 (d, ^3J(H,H) = 8.2 Hz, 2H; bipyridyl-H-3,3′), 7.86 (d, ^3J(H,H) = 8.2 Hz, 2H; bipyridyl-H-4,4′), 5.57–4.26 (m, 54H, OH-2,3,6; H-1), 4.25–2.58 (m, 88H, H-2,3,4,5,6; –CH$_2$), 2.1 (brs, 2H, NH); IR (cm^{-1}, KBr) 3381, 2924, 1704, 1653, 1558, 1463, 1416, 1366, 1240, 1156, 1081, 1030, 943, 848, 756, 707, 608, 579, 530, 440; MS (ESI, H$_2$O/MeOH, 1:1, v/v, m/z): [M+2H]$^{2+}$ calcd 1224.4, found 1224.8, [M+K+H]$^{2+}$ calcd 1243.4, found 1243.8. Anal. calcd for C$_{96}$H$_{150}$N$_4$O$_{68}$·10H$_2$O: C 43.87, H 6.52, N 2.13. Found: C 43.61, H 6.70, N 2.10.

2.1.3. Synthesis of [ZnL(H$_2$O)$_2$(H1)](ClO$_4$)$_2$·10H$_2$O, ZnL(H1)

To an aqueous solution of H1 (0.049 g, 0.020 mmol) was slowly added a solution of slight excessive ZnL(ClO$_4$)$_2$ (0.012 g, 0.024 mmol) in 1 mL H$_2$O with stirring. After ultrasonic oscillation for 30 min, the mixture solution was kept on magnetic stirring for 60 min at room temperature. Then, the white precipitation was formed by diffusing methanol into the mixed aqueous solution. The pure compound was obtained with 45% yield after filter, washed with methanol and dryness with vacuum. Anal. calcd for C$_{113}$H$_{183}$Cl$_2$N$_5$O$_{78}$Zn·10H$_2$O: C 42.73, H 6.44, N 2.21. Found: C 42.63, H 6.35, N 2.38.

2.1.4. Synthesis of [ZnL(H$_2$O)(H2)](ClO$_4$)$_2$·16H$_2$O, ZnL(H2)

The procedure was similar to the above one except that H2 (0.050 g, 0.020 mmol) was used instead of H1. The white pure compound was obtained with 62% yield. MS (ESI, H$_2$O/MeOH, 1:1, v/v, m/z): [ZnL(H2) + 12H$_2$O + 2MeOH]$^{2+}$ calcd 1520.1, found 1520.2; Anal. calcd for C$_{111}$H$_{179}$Cl$_2$N$_7$O$_{77}$Zn·16H$_2$O: C 40.79, H 6.51, N 3.00. Found: C 40.76, H 6.52, N 3.13.

2.2. Potentiometric determination

An automatic titrator (Metrohm 702GPD Titrino) coupled to a Metrohm electrode was used and calibrated according to the Gran method [20,21]. The electrode system was calibrated with buffers and checked by titration of HClO$_4$ with NaOH solution (0.10 M). The thermo-stated cell contained 25 mL of 1.00 mM species in aqueous solutions with the ionic strength maintained at 0.10 M by sodium perchlorate. All titrations were carried out on the aqueous solutions under nitrogen at 298 ± 0.1 K, and initiated by adding fixed volumes of 0.10 M standard NaOH in small increments to the titrated solution. Duplicate measurements were performed, for which the experimental error was below 1%. The titration data were fitted from the raw data with the Hyperquad 2000 program to calculate the ligand protonation constants K_n, the complex formation constant K_{ML}, and the deprotonation constants of the coordinated water pK_a.

2.3. Kinetic study of pNA hydrolysis

The hydrolysis rate of pNA in aqueous solution was measured by following the enhancement in absorption at 400 nm of the p-nitrophenolate released [22–24]. At this wavelength, the absorbance of ester substrate was negligible. The reaction solution was maintained at 298 ± 0.1 K and the ionic strength was adjusted to 0.10 M by sodium perchlorate. 50 mM Tris (pH 7.56–8.56); CHES (pH 8.85–9.49); CAPS (pH 10.03–10.56) were used as buffers. The solutions used in kinetic measurements contained 10% (v/v) CH$_3$CN. Aqueous solutions of the inclusion compounds were prepared by dissolving their compounds in buffer solutions of different pH values and oscillating them ultrasonically for 30 min before testing. The results of the kinetic measurements could be duplicated within ±5%.

## 3. Results and discussion

3.1. Supramolecular assembly

To investigate the assembly of the supramolecular adducts ZnL(H1) and ZnL(H2), two inclusion complexes, [ZnL(H$_2$O)$_2$(H1)] (ClO$_4$)$_2$·10H$_2$O and [ZnL(H$_2$O)(H2)] (ClO$_4$)$_2$·16H$_2$O, were separated by diffusing methanol into the mixed aqueous solution of Bis-CDs and slightly excessive ZnL, and then well-characterized by the elemental analysis. Secondly, ^{1}H NMR spectra were performed to prove the effective encapsulation of the hydrophobic moiety of the Zn(II) complex within the cyclodextrin cavity. In contrast to ZnL, the changes on the chemical shifts of the p-tert-butyl and aromatic protons of L were observed in guest/host inclusion complexes (Fig. S4). In ZnL(H1) and ZnL(H2), the p-tert-butyl protons shift downfield by ca. 0.08 and 0.04 ppm respectively while the aryl proton (H$_b$) shifts upfield by 0.09 and 0.19 ppm respectively, which is similar to an observation on the inclusion complex of β-CD and tert-butyl phenyl group reported by Zubiaur and Jaime [25] and Lucarini et al. [26]. The NMR spectra of ZnL(H1) and ZnL(H2) indicate that the hydrophobic moiety of ZnL binds into the cavity of cyclodextrin.

In addition to the above-mentioned characterized methods, the ESI mass spectroscopy (ESI-MS) was performed to demonstrate the assembly of the supramolecular adducts in solution. When we used ammonium acetate as an additive into the mobile phases of methanol–water [27], a convictive ESI mass spectrum was obtained from Fig. S5. ESI mass spectrum of ZnL(H2) exhibits two clear peaks at m/z = 1225.3 and 1520.2, respectively, which should be assigned to the ligand [H2+2H]$^{2+}$ (calcd. value 1225.1) and the inclusion complex [ZnL(H2)+12H$_2$O+2MeOH]$^{2+}$ (calcd. 1520.1). However, a few peaks only corresponding to H1 (m/z = 1223.6 for [H1+2H]$^{2+}$, calcd. 1223.9) and its solvent adducts were observed for ZnL(H1). The ESI-MS data indicate that ZnL(H2) is a more stable species than ZnL(H1), most possibly due to the presence of an extra coordination between zinc ion of the guest and bipyridyl of the host in ZnL(H2), in addition to hydrophobic interactions.

Furthermore based on the crystal structure of ZnL(β-CD) [14] and the inclusion orientation of tert-butylbenzyl group in a CD cavity reported by Lucarini et al. [26], a conclusion can be drawn that
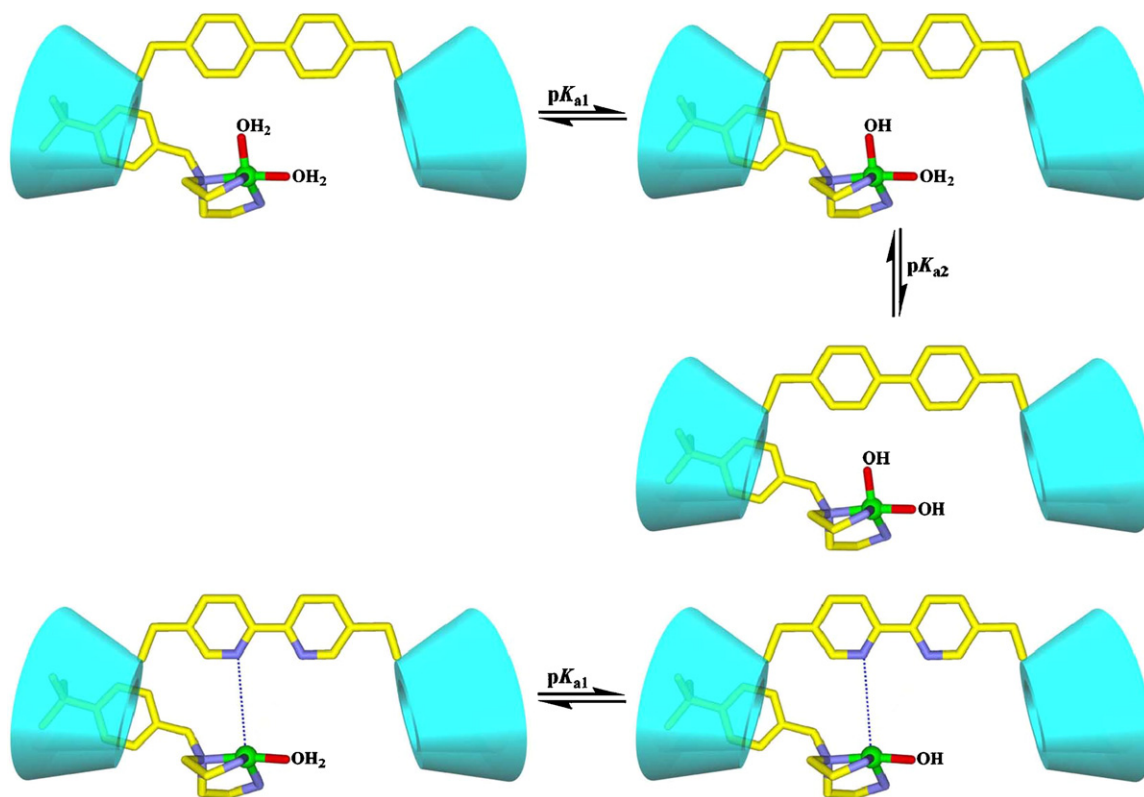
Scheme 3. Deprotonation equilibria of Zn(II)-coordinated water of ZnL(**H1**) and ZnL(**H2**) in aqueous solution.

the *p-tert*-butyl moiety of ZnL binds into the cavity of BisCDs from the primary side of cyclodextrin.

3.2. Complexation and deprotonation constants and species distribution

The protonation constants (K_n) of the ligand and their formation constants (K_{ML}) of inclusion complex and the deprotonation constant (pK_a) of the coordinated water molecule (Scheme 3) as well as species distribution in solution were determined by pH potentiometric titration in $I = 0.1$ M NaClO$_4$ at 298 ± 0.1 K. The pH profiles of the titration curves (Fig. S6) were analyzed by Hyperquad 2000 program [28]. The calculated distribution of the Zn(II) species as a function of pH are shown in Fig. 1 and the equilibrium constants are summarized in Table 1. The addition of NaClO$_4$ does not cause spectral changes, which confirmed that the remaining coordination sites of Zn^{2+} are occupied by water molecules [14].

In **H1** system, it is indicated in Fig. 1a that ZnL(**H1**)H$_2$$^{4+}$, ZnL(**H1**)H^{3+}, ZnL(**H1**)$^{2+}$, ZnL(**H1**)(OH)$^{+}$ and ZnL(**H1**)(OH)$_2$, are involved in the complex formation in pH 2–11, whose species correspond to Eqs. (1)–(5) in Table 1, respectively. As shown in Fig. 1b for **H2** system, ZnL(**H2**)H$_3$$^{5+}$, ZnL(**H2**)H$_2$$^{4+}$, ZnL(**H2**)H^{3+}, ZnL(**H2**)$^{2+}$, and ZnL(**H2**)(OH)$^{+}$, are involved in the complex formation in the same pH range. Compared to in **H1** system, species ZnL(**H2**)(OH)$_2$ disappears whereas species ZnL(**H2**)H$_3$$^{5+}$ emerges in **H2** system, suggesting that nitrogen atoms of the bipyridyl bridge group of **H2** system would bind a proton at low pH and coordinate to the zinc ion at high pH respectively. It is due to this reason that interaction between the guest and the host is enhanced so that the ESI-MS peak of ZnL(**H2**) can be observed. Therefore, the species assigned to Eqs. (6)–(10) in Table 1, respectively.

As shown in Table 1, in the presence of both **H1** and **H2**, the formation constants (K_{ML}) of the guest are higher than that of pure Zn(dien) (a simple analogue of ZnL, dien = diethylenetriamine)
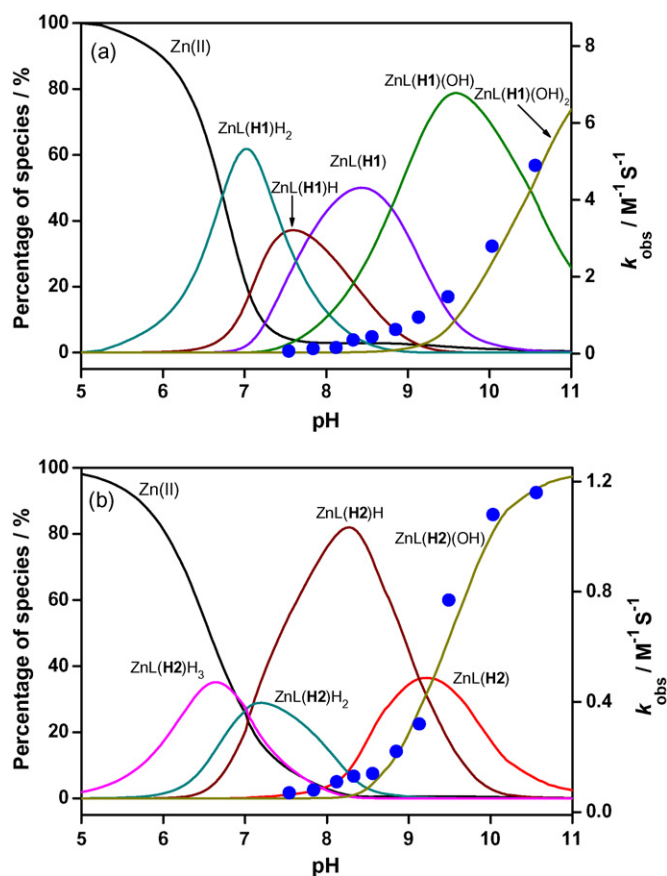

Fig. 1. Distribution plots of species (solid line, left *y*-axis) and k_{obs} values for pNA hydrolysis (●, right *y*-axis) as a function of pH at 0.1 M NaClO$_4$ and 298 ± 0.1 K. (a) 1.00 mM ZnL(**H1**). (b) 1.00 mM ZnL(**H2**).

Table 1
Equilibrium constants of metal complexation in the presence of **H1** and **H2**.

Chemical equilibrium		Equilibrium constant	
$[Zn(H_2O)_6]^{2+} + L + \mathbf{H1} + 2H^+ = [Zn(L)(H_2O)_2(\mathbf{H1})H_2]^{4+} + 4H_2O$	(1)	$\log K_{ZnL(\mathbf{H1})H_2}$	7.68 ± 0.03
$[Zn(H_2O)_6]^{2+} + L + \mathbf{H1} + H^+ = [Zn(L)(H_2O)_2(\mathbf{H1})H]^{3+} + 4H_2O$	(2)	$\log K_{ZnL(\mathbf{H1})H}$	7.94 ± 0.01
$[Zn(H_2O)_6]^{2+} + L + \mathbf{H1} = [Zn(L)(H_2O)_2(\mathbf{H1})]^{2+} + 4H_2O$	(3)	$\log K_{ZnL(\mathbf{H1})}$	10.39 ± 0.04
$[Zn(L)(H_2O)_2(\mathbf{H1})]^{2+} = [Zn(L)(H_2O)(OH)(\mathbf{H1})]^+ + H^+$	(4)	$pK_{a1(ZnLOH(\mathbf{H1}))}$	8.68 ± 0.03
$[Zn(L)(H_2O)(OH)(\mathbf{H1})]^+ = [Zn(L)(H_2O)_2(\mathbf{H1})] + H^+$	(5)	$pK_{a2(ZnL(OH)_2(\mathbf{H1}))}$	10.50 ± 0.04
$[Zn(H_2O)_6]^{2+} + L + \mathbf{H2} + 3H^+ = [Zn(L)(H_2O)_2(\mathbf{H2})H_3]^{5+} + 4H_2O$	(6)	$\log K_{ZnL(\mathbf{H2})H_3}$	7.12 ± 0.03
$[Zn(H_2O)_6]^{2+} + L + \mathbf{H2} + 2H^+ = [Zn(L)(H_2O)(\mathbf{H2})H_2]^{4+} + 5H_2O$	(7)	$\log K_{ZnL(\mathbf{H2})H_2}$	7.22 ± 0.04
$[Zn(H_2O)_6]^{2+} + L + \mathbf{H2} + H^+ = [Zn(L)(H_2O)(\mathbf{H2})H]^{3+} + 5H_2O$	(8)	$\log K_{ZnL(\mathbf{H2})H}$	9.25 ± 0.03
$[Zn(H_2O)_6]^{2+} + L + \mathbf{H2} = [Zn(L)(H_2O)(\mathbf{H2})]^{2+} + 5H_2O$	(9)	$\log K_{ZnL(\mathbf{H2})}$	11.84 ± 0.05
$[Zn(L)(H_2O)(\mathbf{H2})]^{2+} = [Zn(L)(OH)(\mathbf{H2})]^+ + H^+$	(10)	$pK_{a1(ZnLOH(\mathbf{H2}))}$	9.38 ± 0.02

($\log K = 8.92 \pm 0.01$ [29], 10.39 ± 0.04 and 11.84 ± 0.05 for Zn(dien), ZnL(**H1**) and ZnL(**H2**), respectively). The enhancement of complex constant is probably contributed to the effect of both the hydrophobic environment around the coordinated water molecule and weak interactions after complexation, similar to those in carbonic anhydrase or alkaline phosphatase [30]. Moreover, the nitrogen atoms of the bipyridyl bridge of **H2** system possibly coordinates the zinc ion, which results in the K_{ML} of ZnL in **H2** system being higher than that in **H1** system.

Similar to that of $[ZnL(\beta\text{-}CD)(H_2O)_2]^{2+}$ reported previously ($pK_{a1} = 8.20 \pm 0.08$ and $pK_{a2} = 10.44 \pm 0.08$) [14], the first- and second-order pK_a values of coordinated water molecules in $[ZnL(H_2O)_2)(\mathbf{H1})]^{2+}$ were determined to be 8.68 ± 0.03 and 10.50 ± 0.04, respectively. For **H2** system, only one deprotonation constant ($pK_{a1} = 9.38 \pm 0.02$) was determined, which is higher than that of Zn(dien) complex ($pK_a = 8.93$), but is lower than that of Zn(tren) complex ($pK_a = 10.40$, tren = tris[2-(dimethylamino)ethyl]-amine) [31]. This change is probably due to the integrated effect of the bipyridyl-coordination to the zinc ion and the hydrophobic environment provided by the host, which increases and decreases pK_a values respectively.

Other evidences for the different coordination behavior of **H1** and **H2** in the presence of ZnL come from the UV spectra in aqueous solution at 298 ± 0.1 K. Generally, a biphenyl chromophore usually shows one absorption while a bipyridyl chromophore usually shows two absorption peaks in the ultraviolet region [32], and the UV spectra of bipyridyl and its derivatives have previous rationalized [33]. As illustrated in Fig. 2, **H2** shows two absorption peaks at 244 ($\varepsilon = 21{,}006 \, mol^{-1} \, L \, cm^{-1}$) and 292 nm ($\varepsilon = 33{,}894 \, mol^{-1} \, L \, cm^{-1}$), respectively, assigned to absorptions of the bipyridyl chromophore, while **H1** only gives one absorption peak at 261 nm ($\varepsilon = 21{,}160 \, mol^{-1} \, L \, cm^{-1}$), assigned to absorptions of the biphenyl chromophore. A new broad band near 317 nm assigned to ligand to metal charge transfer (LMCT) appears for **H2**, and the absorption at 292 nm decreases in intensity (ε from 33,894 to $33{,}046 \, mol^{-1} \, L \, cm^{-1}$) accompanying a slight bathochromic shift whereas the other absorption peak at 244 nm obviously increases (ε from 21,006 to $22{,}736 \, mol^{-1} \, L \, cm^{-1}$) in the presence of ZnL. These phenomena demonstrate a metal-ligand coordination involving **H2** and zinc ions of ZnL, which is consistent with a ZnN_4O coordination manner of the literatures [34,35]. However, the UV spectrum of ZnL(**H1**) is quite similar to that of its precursor **H1** (Fig. 2b), except for an increase in the intensity at 200–230 nm that can be contributed to the additional absorption of ZnL, in consistent with the fact that biphenyl units in **H1** have no donor-atoms that can coordinate to metal ion. These results will be greatly helpful to understand their binding mode of guest molecules.

3.3. Acetate hydrolysis

The hydrolysis of p-nitrophenyl acetate (pNA) has been used widely for examining catalytic abilities of various models of metallohydrolase [22,36,37]. The initial rate was determined by monitoring formation of the p-nitrophenolate anion at 400 nm, and the initial first-order rate constant (k_{in}) of the total catalyst was calculated as $v/[pNA]_{total}$ [38]. The linear slope of k_{in} vs. [catalyst]$_{total}$ from Eq. (11) resulted in the observed second-order rate constant (k_{obs}).

$$v = k_{in}[pNA] = (k_{obs}[catalyst]_{total} + k_{OH^-}[OH^-] + \cdots)[pNA] \quad (11)$$

As shown in Fig. 3, the plots of k_{in} vs. [catalyst]$_{total}$ were linear within the wide pH range (7.56–10.56). In $[ZnL(\mathbf{H1})]^{2+}$ system, k_{obs} were found to increase exponentially with the increase of pH value (Fig. 1a) in good agreement with the calculated percentage of both $[ZnL(\mathbf{H1})(OH)(H_2O)]^+$ and $[ZnL(\mathbf{H1})(OH)_2]$ species. The result indicates that these hydroxyl complexes are the kinetically active species and thus the kinetic process of pNA hydrolysis is controlled by two acid–base equilibria. Therefore, a complete rate can
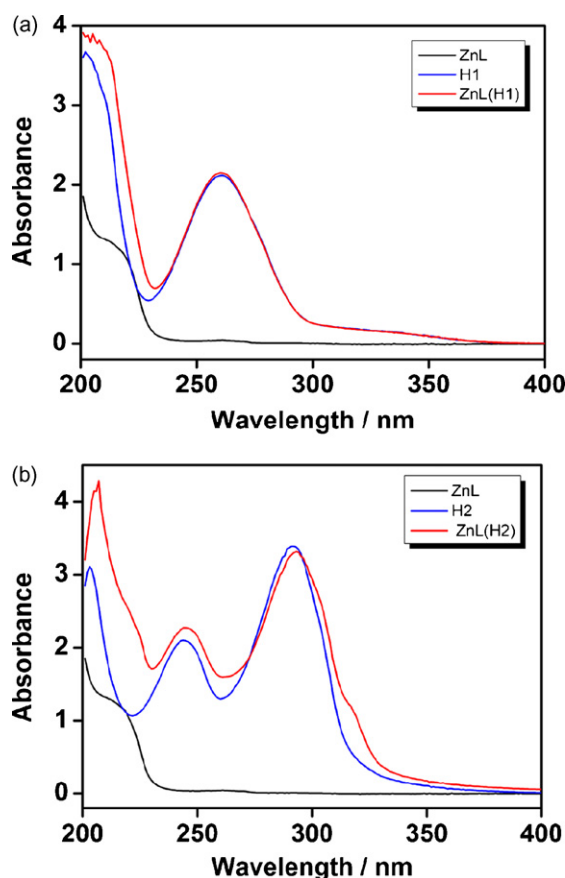

Fig. 2. UV–vis spectra of **H1** (a) and **H2** (b) in the presence of ZnL in aqueous solution at 298 ± 0.1 K. The concentration of all hosts and guests are 1.00×10^{-4} M.
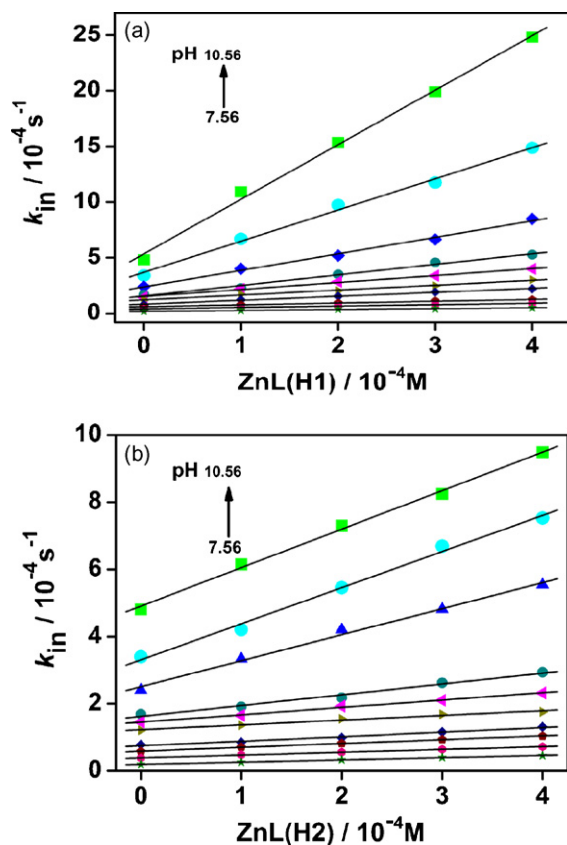
Fig. 3. Dependence of k_{in} on [catalyst]$_{total}$ in pH 7.56–10.56 at 0.1 M NaClO$_4$ and 298 ± 0.1 K in the presence of 10% (v/v) CH$_3$CN. (a) ZnL(**H1**). (b) ZnL(**H2**).

be expressed as Eq. (12).

$$k_{obs} = \frac{k_{cat}^{I}[ZnL(Host)(OH)] + k_{cat}^{II}[ZnL(Host)(OH)_2] + \cdots}{[Zn(II)\,species]_{total}}$$

$$= \frac{k_{cat}^{I}K_{a1}[H^+] + k_{cat}^{II}K_{a1}K_{a2}}{[H^+]^2 + K_{a1}[H^+] + K_{a1}K_{a2}} \quad (12)$$

By fixing pK_a values from pH titration (8.68 and 10.50) for [ZnL(**H1**)]$^{2+}$ two second-order rate constants (k_{cat}) were calculated by curve-fitting from Eq. (12) to be 0.98 M^{-1} s^{-1} (k_{cat}^{I}) for [ZnL(**H1**)(OH)]$^+$ and 8.41 M^{-1} s^{-1} (k_{cat}^{II}) for [ZnL(**H1**)(OH)$_2$] ($R = 0.99$), respectively, which are listed in Table 2. In this study, the k_{OH^-} of pNA value (6.81 M^{-1} s^{-1}) was directly obtained by plotting the intercept vs. [OH$^-$], which is within the normal range of the reported values (4.4–14.8 M^{-1} s^{-1}) [22,39].

Table 2
Rate constants of pNA hydrolysis for ZnL(**H1**), ZnL(**H2**) and other reported Zn(II) complexes.

Species	pK_a	k_{cat} (M^{-1} s^{-1})	Reference
[ZnL(β-CD)(OH)]$^+$	8.47	0.59	[14]
[ZnL(OH)]$^{+a}$	8.74	0.934	[41]
[Zn([15]aneN$_3$O$_2$)(OH)]$^{+b}$	8.8	0.6	[42]
[ZnL(**H1**)(OH)]$^+$	8.68	0.98	This work
[ZnL(**H1**)(OH)$_2$]	10.50	8.41	This work
[ZnL(**H2**)(OH)]$^+$	9.38	1.26	This work
[Zn$_2$L(OH)]$^{3+c}$	7.85	0.35	[40]
[Zn$_2$([15]aneN$_3$O$_2$)(OH)$_2$]$^{2+b}$	9.2	1.3	[42]
[Zn$_3${([14]aneN$_4$)$_3$-tren}(OH)$_2$]$^{4+d}$	8.05	0.34	[43]

[a] L = N,N′-dialkyl-1,10-phenanthroline-2,9-dimethanamine.
[b] [15]aneN$_3$O$_2$ = 1,4-dioxa-7,10,13-triazacyclopentadecane.
[c] L = 1,4,710,19,22,25,28-octaza-13,16,31,34-tetraoxacytriacontane.
[d] ([14]aneN$_4$)$_3$-tren = tris(2-(1,4,8,12-tetraazacyclododecane)ethyl)amine.
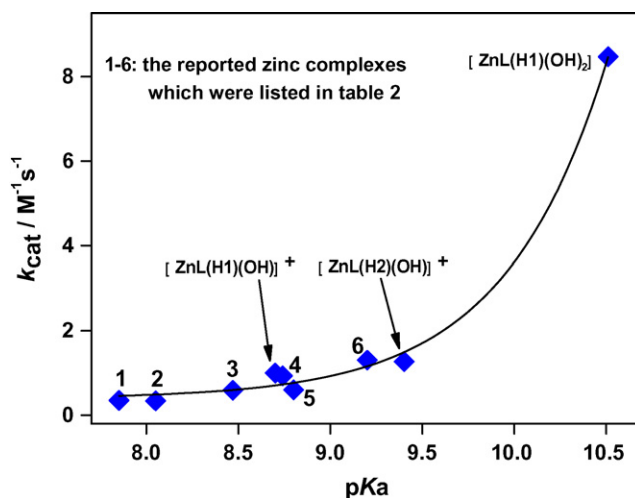

Fig. 4. Dependence on pK_a of the second-order rate constants for pNA hydrolysis catalyzed by the zinc complexes.

In [ZnL(**H2**)]$^{2+}$ system, however, the second-order rate constant were found to exhibit a sigmoidal change (Fig. 1b), which is in good agreement with the calculated percentage of [ZnL(**H2**)(OH)]$^+$ species. The result indicates that the kinetic process of pNA hydrolysis is controlled only by an acid–base equilibrium. Therefore, the catalytic second-order rate constant k_{cat} of [ZnL(**H2**)(OH)]$^+$ can be expressed as the following equation:

$$k_{obs} = \frac{k_{cat}[ZnL(Host)(OH)] + \cdots}{[Zn(II)\,species]_{total}} = \frac{k_{cat}K_a}{[H^+] + K_a} \quad (13)$$

Fixing pK_a values from pH titration (9.38), k_{cat} value (Table 2) was obtained by curve-fitting from Eq. (13) to be 1.26 M^{-1} s^{-1} for [ZnL(**H2**)(OH)]$^+$ ($R = 0.98$).

Dependence on pK_a values of the second-order rate constants for pNA hydrolysis catalyzed by both supramolecular systems and other reported zinc complexes is shown in Fig. 4. The k_{cat}^{I} of the first-order monohydroxyl active species in [ZnL(**H1**)]$^{2+}$ system fell in the normal range with similar pK_a values [40], however, its k_{cat}^{II} of the second-order dihydroxyl form is 100-fold higher than that of its simple unassembled analogue [Zn(dien)(OH)(H$_2$O)]$^+$ ($k_{cat} = 0.08$ M^{-1} s^{-1}) [29], and is also higher than those of other reported binuclear and trinuclear complexes (Table 2) [41–43]. Interestingly, the pK_a dependence of the second-order rate constants for pNA hydrolysis catalyzed by mono- and di-hydroxyl active species and other reported complexes exhibits exponential growth rather than a linear increase (Fig. 4). Therefore, the kinetic behavior of dihydroxyl species cannot be explained simply by reference to changes in pK_a. Based on the structure analysis of ZnL(H$_2$O)$_2$(CD) [14], it can be inferred that the hydroxyl group in an equatorial position of the trigonal bipyramid in ZnL(**H1**) system attacks the carboxylic carbon directly at low pH, and the axial one with higher nucleophilic attack the carboxylic carbon at high pH (Scheme 3). While the p-nitrophenyl group can be included into the cyclodextrin cavity from the primary side by the hydrophobic interaction in aqueous solution [44,45] and the binding manner of ZnL(BCD) [13], it can be drawn the conclusion that the cyclodextrin dimer acts as the catalytic reaction reactor with one cavity fixing the catalyst and the other cavity fixing the substrate. This pre-organized geometry of catalyst/substrate complex is in favor of the nucleophilic attack on the carboxylic carbon with the dihydroxyl species. Moreover, the formation of dihydroxyl species can obviously decrease the positive charge distribution of the whole nucleophilic agent, which is propitious to nucleophilic attack. Also, compared with monohydroxyl species, dihydroxyl species have more nucleophilic groups. There-
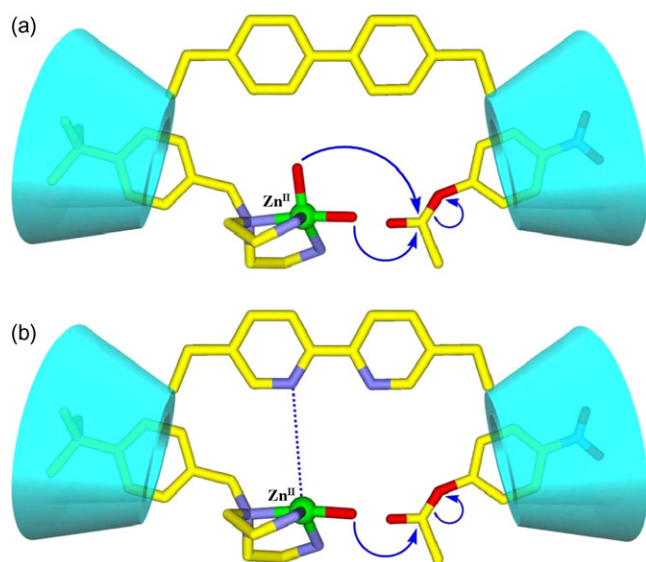
Scheme 4. Suggested intermediate of pNA hydrolysis catalyzed by mimics. (a) ZnL(**H1**). (b) ZnL(**H2**).

fore, [ZnL(**H1**)(OH)$_2$] have a higher catalytic activity for hydrolysis of the carboxylic ester than other zinc complex. As for ZnL(**H2**) system, k_{cat} of the monohydroxyl active species [ZnL(**H2**)(OH)]$^+$ was located in the normal values range of other zinc complexes with the similar pK_a [40]. One important reason is that the coordination between the zinc ion and the bipyridyl bridge takes place at the equatorial position of the trigonal bipyramid in ZnL(**H2**) system according to the spectral data [14,32], and then the coordinated water molecule holds at the axial position. Therefore, the coordinated water has to deprotonate and attack the carboxylic carbon at relatively high pH. A proposed intermediate is shown in Scheme 4.

4. Conclusions

Two new cyclodextrin dimers have been synthesized, characterized and applied as catalyst precursors. One hydrophobic cavity of the dimer is assembled with a Zn^{2+}-triamine complex containing pendant side of 4-*tert*-butylbenzyl to construct a supramolecular metallohydrolase model, in which hydroxyl species acting as active catalysis species can be stabilized by the supramolecular system in solution. Another hydrophobic cavity of the dimer is used to capture the substrate containing a hydrophobic group, such as pNA. Studies on pNA hydrolysis show that ZnL(**H1**) system exhibits the kinetic behavior of dihydroxyl species and has much higher catalytic activity than ZnL(**H2**) system. In such a host–guest system, an original intermolecular reaction between ZnL and pNA is translated into a *quasi*-intramolecular reaction by the cooperative hydrophobic interaction between the two CD cavities and the catalyst/substrate. All kinetics data are well consistent with the thermodynamics analysis.

Acknowledgments

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molcata.2009.03.026.

References

[1] M. Creus, A. Pordea, T. Rossel, A. Sardo, C. Letondor, A. Ivanova, I. LeTrong, R.E. Stenkamp, T.R. Ward, Angew. Chem. Int. Ed. 47 (2008) 1400.
[2] C.M. Thomas, T.R. Ward, Chem. Soc. Rev. 34 (2005) 337.
[3] J. Steinreiber, T.R. Ward, Coord. Chem. Rev. 252 (2008) 751.
[4] B. Zhang, R. Breslow, J. Am. Chem. Soc. 119 (1997) 1676.
[5] R. Villalonga, R. Cao, A. Fragoso, Chem. Rev. 107 (2007) 3088.
[6] J.M. Haider, Z. Pikramenou, Chem. Soc. Rev. 34 (2005) 120.
[7] Y. Liu, Y. Chen, Acc. Chem. Res. 39 (2006) 681.
[8] R. Breslow, L.E. Overman, J. Am. Chem. Soc. 92 (1970) 1075.
[9] Z.Y. Dong, X.Q. Li, K. Liang, S.Z. Mao, X. Huang, B. Yang, J.Y. Xu, J.Q. Liu, G.M. Luo, J.C. Shen, J. Org. Chem. 72 (2007) 606.
[10] F. Sallas, A. Marsura, V. Petot, I. Pinter, J. Kovacs, L. Jicsinszky, Helv. Chim. Acta 81 (1998) 632.
[11] R.R. French, P. Holzer, M.G. Leuenberger, W.D. Woggon, Angew. Chem. Int. Ed. 39 (2000) 1267.
[12] P. Tastan, E.U. Akkaya, J. Mol. Catal. A: Chem. 157 (2000) 261.
[13] Y.H. Zhou, M. Zhao, J.H. Li, Z.W. Mao, L.N. Ji, J. Mol. Catal. A: Chem. 293 (2008) 59.
[14] Y.H. Zhou, H. Fu, W.X. Zhao, M.L. Tong, C.Y. Su, H. Sun, L.N. Ji, Z.W. Mao, Chem. Eur. J. 13 (2007) 2402.
[15] Y.H. Zhou, H. Fu, W.X. Zhao, W.L. Chen, C.Y. Su, H. Sun, L.N. Ji, Z.W. Mao, Inorg. Chem. 46 (2007) 734.
[16] H.F.M. Nelissen, M.C. Feiters, R.J.M. Nolte, J. Org. Chem. 67 (2002) 5901.
[17] L. Jicsinszky, R. Ivanyi, Carbohydr. Polym. 45 (2001) 139.
[18] R.C. Petter, J.S. Salek, C.T. Sikorski, G. Kumaravel, F.T. Lin, J. Am. Chem. Soc. 112 (1990) 3860.
[19] J.C. Rodriguez-Ubis, B. Alpha, D. Plancherel, J.M. Lehn, Helv. Chim. Acta 67 (1984) 2264.
[20] G. Gran, Acta Chem. Scand. 4 (1950) 559.
[21] Z.W. Mao, G. Liehr, R. van Eldik, Dalton Trans. (2001) 1593.
[22] M. Subat, K. Woinaroschy, S. Anthofer, B. Malterer, B. Konig, Inorg. Chem. 46 (2007) 4336.
[23] J. Huang, S.A. Li, D.F. Li, D.X. Yang, W.Y. Sun, W.X. Tang, Eur. J. Inorg. Chem. 2004 (2004) 1894.
[24] K.L. Klinkel, L.A. Kiemele, D.L. Gin, J.R. Hagadorn, J. Mol. Catal. A: Chem. 267 (2007) 173.
[25] M. Zubiaur, C. Jaime, J. Org. Chem. 65 (2000) 8139.
[26] M. Lucarini, E. Mezzina, G.F. Pedulli, Eur. J. Org. Chem. 2000 (2000) 3927.
[27] M.A. Strege, Anal. Chem. 70 (1998) 2439.
[28] P. Gans, A. Sabatini, A. Vacca, Talanta 43 (1996) 1739.
[29] T. Itoh, Y. Fujii, T. Tada, Y. Yoshikawa, H. Hisada, B. Chem. Soc. Jpn. 69 (1996) 1265.
[30] W.N. Lipscomb, N. Strater, Chem. Rev. 96 (1996) 2375.
[31] G. Anderegg, V. Gramlich, Helv. Chim. Acta 77 (1994) 685.
[32] Y. Liu, X.Q. Li, Y. Chen, X.D. Guan, J. Phys. Chem. B 108 (2004) 19541.
[33] K. Nakamoto, J. Phys. Chem. 64 (1960) 1420.
[34] D. Li, S. Li, D. Yang, J. Yu, J. Huang, Y. Li, W. Tang, Inorg. Chem. 42 (2003) 6071.
[35] S. Aoki, M. Zulkefeli, M. Shiro, M. Kohsako, K. Takeda, E. Kimura, J. Am. Chem. Soc. 127 (2005) 9129.
[36] J.D. Caro, P. Rouimi, M. Rovery, Eur. J. Biochem. 158 (1986) 601.
[37] N. Saki, E.U. Akkaya, J. Mol. Catal. A: Chem. 219 (2004) 227.
[38] J. Suh, S.J. Son, M.P. Suh, Inorg. Chem. 37 (1998) 4872.
[39] W.P. Jencks, M. Gilchrist, J. Am. Chem. Soc. 90 (1968) 2622.
[40] A.K. Yatsimirsky, Coord. Chem. Rev. 249 (2005) 1997.
[41] X.C. Su, H.W. Sun, Z.F. Zhou, H.K. Lin, L. Chen, S.R. Zhu, Y.T. Chen, Polyhedron 20 (2001) 91.
[42] C. Bazzicalupi, A. Bencini, A. Bianchi, V. Fusi, C. Giorgi, P. Paoletti, B. Valtancoli, D. Zanchi, Inorg. Chem. 36 (1997) 2784.
[43] C. Bazzicalupi, A. Bencini, E. Berni, C. Giorgi, S. Maoggi, B. Valtancoli, Dalton Trans. (2003) 3574.
[44] R. Breslow, C. Schmuck, J. Am. Chem. Soc. 118 (1996) 6601.
[45] R. Breslow, N. Nesnas, Tetrahedron Lett. 40 (1999) 3335.