Catalytic Hydrolysis of Phosphate Diester with Metal Complexes of Macrocyclic Tetraamine in Comicellar Solution

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Four novel pyridine or benzene ring-containing pendant macrocyclic dioxotetraamines 2,6-dioxo-1,4,7,10-tetraazacyclododecane ligands have been synthesized. Their metal complexes have been investigated as catalysts for the hydrolysis of bis(*p*-nitrophenyl) phosphate (BNPP) in aqueous comicellar solution. The results indicate that the hydrophobic interaction between substrate and metal complex, the nature of transition metal ion, and the micellar microenvironment are important factors for the hydrolysis of BNPP. Large rate enhancement (up to over two-three orders of magnitude) employing 5 has been observed.

Keywords Macrocyclic polyamine, metal complex, phosphate diester, catalytic hydrolysis, micelle

Introduction

Macrocyclic ligands are thermodynamically more stable and more selective metal ion binders than their open-chain analogues. The catalytic hydrolysis of phosphate diesters by metal complexes of macrocyclic tetraamines (cyclen, 1,4,7,10-tetraazacyclododecane) in aqueous solution or micellar solution has been intensively studied.[1-3] The hydrolysis of phosphate diester bonds is the model of the cleavage of the phosphate diester bonds of DNA and RNA, and it is essential for further developments in biotechnology, molecular biology therapy and related fields.[4] Although phosphate diester bonds are highly stable kinetically at physiological conditions, model studies have shown that metallomicelles made up of ligand surfactants (or lipophilic ligands) chelating metal ions are able to promote the cleavage of phosphoric and carboxylic esters or amides.[5-7] Recently, our group discovered that metal complexes of chiral lipophilic pyridyl-containing β-amino alcohol ligands are very efficient for the enantioselective hydrolysis of α-amino acid ester in chiral metallomicelles,[8a] dimeric estradiol enzyme model containing imidazolyl[8b] and imidazolium-bridged cyclodextrin dimers could also catalyze the hydrolysis of carboxylates and phosphates.[8c] We now report the synthesis of four novel pyridine or benzene ring-containing pendant macrocyclic tetraamine ligands and the catalytic hydrolysis of bis(*p*-nitrophenyl)phosphate (BNPP) by their metal complexes in aqueous comicellar solution.

Results and discussion

Ligands

Ligands **5** and **7** were synthesized according to the procedures outlined in Scheme 1. Compound **1** was selectively mono-*O*-alkylated to **2**, and then **2** was converted to **3** by treatment with $SOCl_2$. Compound **6** was obtained by refluxing **1a** with 48% HBr. Ligands **5** and **7** were obtained by coupling of **3** and **6** with 2,6-dioxo-1,4,7,10-tetraazacyclododecane (**4**), respectively. Their structures were confirmed by elemental analysis, IR, MS, and ^{1}H NMR.

Ligands **5** and **7** contain rigid pyridine or benzene ring. Ligands **5a** and **5c** contain hydrophobic long chains, while **5b** and **7** do not. They have been investi-

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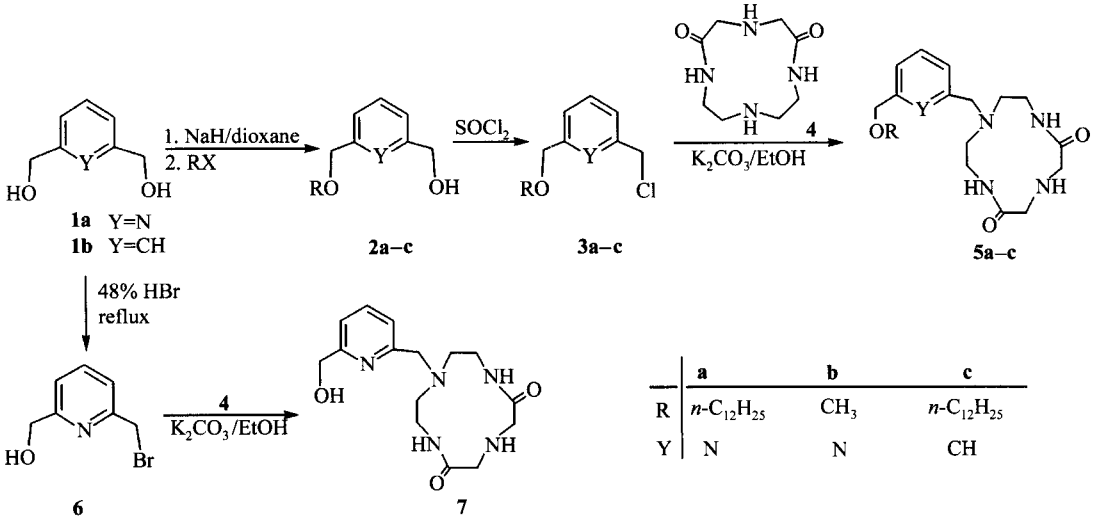
gated so that their catalytic activities can be compared.

Lipophilic ligands **5a** and **5c** are barely soluble in neutral water, ligands **5b** and **7** are soluble in water. Their clear solutions, free or as transition metal complexes, can be obtained only in the presence of micelles of inert surfactants such as polyethylene glycol dodecyl ether(Brij35) as a matrix of comicellar aggregates. The kinetic experiments were carried out using mixed micelles composed of a metal complex and a cosurfactant.

Scheme 1



Kinetics

The rate of hydrolysis was followed under pseudo-first-order conditions by observing the release of *p*-nitrophenol spectrophotometrically. The pseudo-first-order constants (k_{obs}) for the cleavage of BNPP catalyzed by metal complexes comicellized with Brij35 are summarized in Table 1. The results show that large rate enhancements were observed only in the presence of both the ligand and metal ion, and the catalytic activity is the result of synergistic cooperation of the ligand and metal ion. The catalytic activity shows also a distinct dependence on the nature of the transition metal ion, and has the order of $Co^{2+} > Zn^{2+} > Cu^{2+} > Ni^{2+}$. Although both **5a** and **5c** have the same lipophilic long chain, ligand **5a** exhibits larger activity compared to ligand **5c**. The result is caused probably by the participation of nitrogen atom of the pyridine in the intermediate.

Table 2 shows the kinetic data observed for the cleavage of BNPP in the presence of anionic *n*-dodecyl sodium sulfate (SDS), cationic *n*-hexadecyltrimethylammonium bromide (CTABr), or nonionic polyethylene glycol dodecyl ether (Brij35) as the matrix

Table 1 Pseudo-first-order rate constants (k_{obs}, s^{-1}) for the hydrolysis of BNPP by ligands **5**, **7** and M^{2+} comicellized with Brij35

Entry	Ligand	M^{2+}	$k_{obs} \times 10^{-3}$ (s^{-1})
1	none	Cu^{2+}	8.09
2	none	Zn^{2+}	6.90
3	**5a**	Cu^{2+}	398
4	**5a**	Zn^{2+}	621
5	**5b**	None	3.49
6	**5b**	Cu^{2+}	3910
7	**5b**	Zn^{2+}	4490
8	**5b**	Ni^{2+}	3230
9	**5b**	Co^{2+}	4970
10	**5c**	Cu^{2+}	275
11	**5c**	Ni^{2+}	309
12	**7**	None	8.77
13	**7**	Cu^{2+}	2720
14	**7**	Zn^{2+}	3570
15	**7**	Ni^{2+}	2320
16	**7**	Co^{2+}	3750

Conditions: 35 ± 0.1℃, pH 7.41 [0.02 mol·dm^{-3}, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES) buffer], [Ligand] = 2.5 × 10^{-4} mol·dm^{-3}, [Substrate] = 2.5 × 10^{-3} mol·dm^{-3}, [M^{2+}] = 2.5 × 10^{-4} mol·dm^{-3}, [Brij35] = 2.0 × 10^{-3} mol·dm^{-3}.

of comicellar aggregates. In the Brij35 micelle the catalytic activity induced by **5b**-Cu^{2+} is higher than in CTABr and SDS. These observations indicate that the micellar microenvironment is of importance for the activity.

Table 2 Pseudo-first-order rate constants (k_{obs}, s^{-1}) for the hydrolysis of BNPP by **5b**-Cu^{2+} comicellized with the different cosurfactant

Entry	Ligand	Cosurfactant	$k_{obs} \times 10^{-3}$ (s^{-1})
1	None	Brij35	8.09
2	**5b**	Brij35	3910
3	**5b**	SDS	376
4	**5b**	CTABr	298

Conditions: [Cosurfatant] = 2.0×10^{-3} mol·dm^{-3}, [Cu^{2+}] = 2.5×10^{-4} mol·dm^{-3} (see Table 1 for other conditions).

Stoichiometry of the reactive complexes

In order to determine the stoichiometry of kinetically reactive complexes, the kinetic version of Job plots was examined by plotting k_{obs} as a function of molar fraction of ligand (γ), keeping the total concentrations of the ligand and metal ion constant. The results show in Fig. 1 that in the case of Cu^{2+} and ligand **5b** the rate maximum is observed at $\gamma = 0.5$, which corresponds to a stoichiometry of ligand:Cu^{2+} = 1:1. Ligand **5b** forms stable complexes with Cu^{2+} as indicated by the sharp maximum in the Job plots.

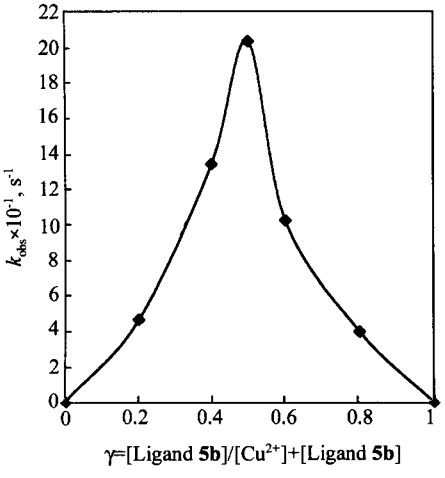


Fig. 1 Kinetic Job plots for the hydrolysis of BNPP by ligand **5b**-Cu^{2+} in HEPES buffer (0.01 mol·dm^{-3}), pH 6.93, 35± 0.1℃, [**5b**] + [Cu^{2+}] = 5.0×10^{-4} mol·dm^{-3} (see Table 1 for other conditions).

Mechanism

The above kinetic behavior of **5b**-M^{2+}-catalyzed hydrolysis of BNPP is outlined in Scheme 2 on the basis of previous reports.[2b,2c,9] The possible mechanism is based on a pentacoordinated or hexacoordinated geometry of the metal complexes although for Cu^{2+}, Zn^{2+}, Ni^{2+}, and Co^{2+} alternative forms are possible.

The metal complex may be represented as **A** where M^{2+} coordinates up to five donors in a pentacoordinated geometry, the four nitrogen atoms of the macrocycle occupy the strongest position and the oxygen atom of the water occupies the top position. The coordinated water is activated by the metal ion which provides the effective nucleophile at near neutral pH, and a pseudo-intermolecular nucleophilic attack of the activated hydroxyl group on the P = O of the substrate as **B** results in the expulsion of *p*-nitrophenol. The formation of ternary complex **B** is the key step of the hydrolysis. Because the pyridine ring is the rigid structure, therefore the screening action of the pyridine group is important to the formation of **B**. When R group is long chain, the steady of **B** is poorer, the rate of hydrolysis is reduced. Then the other water coordinated by M^{2+} and the oxygen of the phosphate form the intermediate **C**. The intermediate is hydrolyzed to release monophosphate ester and regenerate the catalytic species by attack of the free or metal-ion bonded hydroxide ion on P = O of coordinated ester group, thus defining the catalytic cycle.

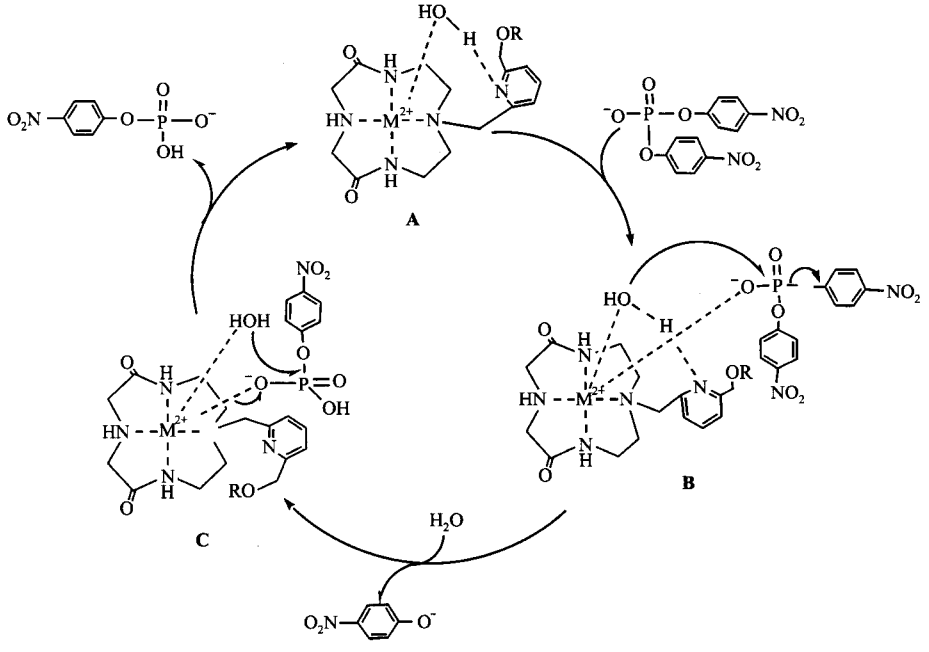
Experimental

General methods and material

Melting points were taken on a micro-melting apparatus and are uncorrected. ^1H NMR spectra were recorded at 90 MHz and 400.13 MHz, and chemical shifts are reported relative to internal Me_4Si. Mass spectral data were recorded on a Finnigan MAT 4510 spectrometer. Elemental analyses were performed with a Carlo-Elba 1106 instrument. IR spectra in cm^{-1} were recorded on a Perkin-Elmer 16PC spectrometer. Kinetic runs were conducted on a Perkin-Elmer Lambda 4B UV/Vis spectrophotometer equipped with a thermostated cell compartment. $Zn(ClO_4)_2 \cdot 6H_2O$, $Cu(ClO_4)_2 \cdot 6H_2O$, $Ni(CH_3COO)_2 \cdot 4H_2O$, $Co(CH_3COO)_2 \cdot 4H_2O$, *n*-dodecyl sodium sulfate (SDS), *n*-hexadecyltrimethyl-ammonium

bromide (CTABr), polyethylene glycol dodecyl ether (Brij35) and bis(*p*-nitrophenyl) phosphate (BNPP) were purchased from commercial sources and used without further purification. The buffer was 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES) ($pK_a = 7.5$). The following compounds were prepared according to literature procedures: 2, 6-bis(hydroxymethyl)pyridine (**1a**),[10] 2-(bromomethyl)-6-(hydroxymethyl)pyridine (**6**),[10] 2,6-dioxo-1,4,7,10-tetraazacyclododecane (dioxo[12] N_4, **4**).[11] 1,3-Bis(hydroxymethyl) benzene (**1b**) was prepared by reduction of *m*-phathalic acid diester with $LiAlH_4$. Compounds **2c** and **3c** were prepared according to synthetic method of compound **2a** or **3a**.[8a] Tetrahydrofuran and dioxane were purified according to the standard methods. All other chemicals and reagents were obtained commercially and used without further purification.

Scheme 2



1-(*n-Dodecoxymethyl*)-3-(*hydroxymethyl*) *benzene* (**2c**)

The crude material purified by column chromatography (SiO_2, $CHCl_3$:CH_3OH = 12:1) to give a pale yellow oil. The resulting oil was recrystallized from petroleum (b.p. 30—60℃) to give white needles (yield 75.0%). m.p. 44—46℃. ^1H NMR (90 MHz, $CDCl_3$) δ: 0.91 (t, 3H, CH_3), 1.25 (s, 18H, $(CH)_9CH_3$), 1.63—1.67 (m, J = 2.4 Hz, 2H, $OCH_2CH_2(CH_2)_9$), 3.45 (t, J = 6.7 Hz, 2H, $OCH_2(CH_2)_{10}$), 4.50 (s, 2H, $PhCH_2OC_{12}H_{25}$), 4.70 (s, 2H, $PyCH_2OH$), 7.20—7.24 (d, J = 9.5 Hz, 2.8Hz, 3H, PhH_1, PhH_3, PhH_5), 7.30 (d, J = 9.5 Hz, 1H, PhH_2); MS m/z(%): 305 ($M^+ - 1$, 30).

1-(*n-Dodecoxymethyl*)-3-(*chloromethyl*) *benzene* (**3c**)

The crude purified to give white solid by column chromatography (SiO_2, petroleum: ethyl acetate = 5:1) (yield 88.1%). ^1H NMR (400 MHz, $CDCl_3$) δ: 0.90 (t, J = 7.0 Hz, 3H, CH_3), 1.26 (s, 16H, $(CH_2)_8CH_3$), 1.60—1.65 (m, J = 2.4 Hz, 4H, $OCH_2CH_2(CH_2)_8$), 3.47 (t, J = 6.9 Hz, 2H, $OCH_2(CH_2)_{10}$), 4.50 (s, 2H, $PhCH_2O$), 4.59 (s, 2H, $PhCH_2Cl$), 7.26 (d, J = 9.5 Hz, 1H, PhH_5), 7.33—7.37 (d, J = 9.5, 2.8 Hz, 2H, PhH_4, PhH_6), 7.42 (d, 1H, PhH_2). MS m/z(%): 326 ($M^+ + 1$, 45).

General procedure for the synthesis of ligands **5** *and* **7**

To a stirred mixture of K_2CO_3 (0.28 g), KI (0.3 g) and 2, 6-dioxo-1, 4, 7, 10-tetraazacyclododecane **4** (0.4 g, 2.0 mmol) in 30 mL of dry ethanol, the corresponding compound **3** or **6** (2.0 mmol) dissolved in 30 mL of dry ethanol was added dropwise at reflux under N_2 atmosphere. After the addition was completed, the mixture was kept at this temperature for 5 h. The slurry was filtered, the organic solvent was evaporated under reduced pressure, and the residue was chromatographed on silica gel ($CHCl_3$:CH_3OH = 10:1).

10-[6-(*n-Dodecoxymethyl*) *pyridine-2-yl*] *methyl-2, 6-dioxo-1,4,7,10-tetraazacyclododecane* (**5a**)

White solid (yield 46.5%); m.p 102—104℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.86 (t, J = 6.9 Hz, 3H, CH_3), 1.19 (s, 18H, $(CH_2)_9CH_3$), 1.60—1.67 (m, J = 2.4 Hz, 2H, $OCH_2CH_2(CH_2)_9$), 2.83 (b, 4H, CH_2NCH_2), 3.30 (b, 4H, $2CH_2NHCO$), 3.40 (s, 4H, $2CH_2CO$), 3.50 (t, J = 6.6 Hz, 2H, OCH_2-$(CH_2)_{10}$), 3.52 (t, J = 2.5 Hz, 1H, CH_2NHCH_2), 3.89 (s, 2H, $PyCH_2N$), 4.65 (s, 2H, $PyCH_2O$), 7.21—7.38 (d, J = 7.5 Hz, 2H, PyH_3, PyH_5), 7.71 (d, J = 7.5 Hz, 1H, PyH_4), 8.29 (b, 2H, $2CH_2NHCO$). IR (KBr) ν: 3358, 3065, 2980, 2950, 1654, 1056 cm^{-1}. Anal. Calcd. for $C_{27}H_{47}N_5O_3$: C, 66.22, H, 9.67, N, 14.30; Found: C 66.10, H 9.70, N 14.60. MS m/z(%): 490(M^+ + 1, 15).

10-[6-*Methoxymethyl*) *pyridine-2-yl*] *methyl-2,6-dioxo-1,4,7,10-tetraazacyclododecane* (**5b**)

White solid (yield 47.8%); m.p. 126—127℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.80 (b, 4H, CH_2NCH_2), 3.28 (b, 4H, $2CH_2NHCO$), 3.40 (s, 4H, $2CH_2CO$), 3.46 (s, 3H, CH_3), 3.50 (s, 1H, CH_2NHCH_2), 3.88 (s, 2H, $PyCH_2N$), 4.61 (s, 2H, $PyCH_2O$), 7.15—7.34 (d, J = 7.6 Hz, 2H, PyH_3, PyH_5), 7.69 (d, J = 7.6 Hz, 1H, PyH_4), 8.25 (b, 2H, $2CH_2NHCO$). IR (KBr) ν: 3311, 3054, 2980, 2835, 1656, 1052 cm^{-1}. Anal. Calcd for $C_{16}H_{25}N_5O_3$: C 57.29, H 7.51, N 20.88; Found: C 57.40, H 7.41, N 21.10. MS m/z(%): 336(M^+ + 1, 50).

10-[3-(*n-Dodecoxymethyl*)] *benzyl-2,6-dioxo-1,4,7,10-tetraazacyclododecane* (**5c**)

White solid (yield 30.7%): m.p. 96—98℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.91 (t, 3H, CH_3), 1.25 (s, 18H, $(CH_2)_9CH_3$), 1.61—1.68 (m, J = 2.4 Hz, 2H, $OCH_2CH_2(CH_2)_9$), 2.66 (s, 4H, CH_2NHCH_2), 3.29 (b, 4H, $2CH_2NCO$), 3.43 (s, 4H, $2CH_2CO$), 3.46 (t, J = 6.7 Hz, 2H, OCH_2-$(CH_2)_{10}$), 3.48 (t, J = 2.5 Hz, 1H, CH_2NHCH_2), 3.71 (s, 2H, $PhCH_2N$), 4.50 (s, 2H, $PhCH_2O$), 7.21 (d, J = 9.6 Hz, 2H, PhH_1, PhH_3), 7.26 (d, J = 9.6 Hz, 1H, PhH_5), 7.32 (d, J = 9.6 Hz, 1H, PhH_2), 7.41 (b, 2H, $2CH_2NHCO$). IR (KBr) ν: 3310, 3022, 2920, 2852, 1656, 1536, 1430, 1068, 794, 710 cm^{-1}. Anal. Calcd. for $C_{28}H_{48}N_4O_3$: C 68.81, H 9.90, N 11.46; Found: C 68.71, H 9.95, N 11.61. MS m/z(%): 488(M^+, 10).

10-[6-(*Hydromethyl*) *pyridine-2-yl*] *methyl-2,6-dioxo-1,4,7,10-tetraazacyclododecane* (**7**)

White solid (yield 53%); m.p. 144—145℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.30 (s, 1H, CH_2OH), 2.74 (t, J = 3.2 Hz, 4H, CH_2NHCH_2), 3.27 (b, 4H, $2CH_2NHCO$), 3.47 (s, 4H, $2CH_2CO$), 3.50 (s, 1H, CH_2NHCH_2), 3.86 (s, 2H, $PyCH_2N$), 4.79 (s, 2H, $PyCH_2O$), 7.18—7.26 (d, J = 7.7 Hz, 2H, PyH_3, PyH_5), 7.71 (d, J = 7.7 Hz, 1H, PyH_4), 7.99 (b, 2H, $2CH_2NHCO$). Anal. Calcd. for $C_{15}H_{23}$-N_5O_3: C 56.06, H 7.21, N 21.79; Found: C 55.96, H 7.34, N 21.91. MS (m/z): 322(M^+ + 1, 25).

Kinetic studies

Solutions of the ligands, metal ions and consurfactants were prepared in HEPES buffer (0.02 mol·dm^{-3}). The reaction temperature was maintained at 35 ± 0.1℃. Kinetics were typically started by injecting an acetonitrile solution (10%, W/V) of substrate (BNPP) into a 1 cm cuvette containing 3.0 mL of buffered micellar solution and the desired concentration of metal ion and ligand. Pseudo-first-order rate constants (k_{obs}) for the hydrolysis of BNPP were determined by monitoring the release of p-nitrophenol at 400 nm (pH 6.3—8.5). The UV absorption increase was recorded immediately. The

rate constants for each reaction were determined three times from three separate runs with an uncertainty of less than 5%.

References

1 (a) Lindoy, L. F. *The Chemistry of Macrocyclic Ligand Complexes*, Cambridge University Press: Cambridge, U.K., **1989**.
(b) Chin, K. O. A.; Morrow, J. R. *Inorg. Chem.* **1991**, *30*, 4295.
(c) Chin, K. O. A.; Morrow, J. R.; Lake, C. H.; Churchill, M. R. *Inorg. Chem.* **1994**, *33*, 656.
(d) Amin, S.; Voss, J. D. A.; Dew, W.; Horrocks, J.; Morrow. J. R. *Inorg. Chem.* **1996**, *35*, 7466.

2 (a) Kimura, E.; Kodama, Y.; Koike, T. *J. Am. Chem. Soc.* **1995**, *117*, 8304.
(b) Shiranoya, M.; Ikeda, T.; Kimura, E.; Shiro, M. *J. Am. Chem. Soc.* **1994**, *115*, 3848.
(c) Kimura, E.; Hashimoto, H.; Koike. T. *J. Am. Chem. Soc.* **1996**, *118*, 10963.

3 Hettich, R.; Schneider, H. J. *J. Am. Chem. Soc.* **1997**, *119*, 5638.

4 (a) Komiyama, M.; Sumaoka, J. *Curr. Opin. Chem. Biol.* **1998**, *2*, 751.
(b) Oivanen, M.; Kuusela, S.; Lõ nnberg, H. *Chem. Rev.* **1998**, *98*, 961.
(c) Prativel, G.; Bernadow, J.; Meuniere, B. In *Advances in Inorganic Chemistry*, Vol. 45, Ed.: Sykes, A. G., Academic Press: San Diego, **1998**, p.251.
(d) Chin, J. *Curr. Opin. Chem. Biol.* **1997**, *1*, 514.
(e) Komiyama, M.; Takeda, N.; Shigekawa, H. *Chem. Commun.* **1999**, 1443.
(f) Woiflenden, R.; Ridgway, C.; Young, G. *J. Am. Chem. Soc.* **1998**, *120*, 833.

5 Bhattacharya, S.; Shahalatha, K.; Geogre, S. *J. Org. Chem.* **1998**, *63*, 27.

6 (a) Weijen, T. G. J.; Engbersen. J. F. J. *Recl. Trav. Chim. Pays-Bas* **1993**, *112*, 351.
(b) Scrimin, P.; Tecilla, P.; Tonellato, U. *J. Org. Chem.* **1991**, *56*, 161.
(c) Bunton, C. A.; Scrimin, P.; Tecilla, P. *J. Chem. Soc., Perkin Trans.* **1996**, *2*, 419.

7 Lim, Y. Y.; Tan, E. H. L.; Can, L. H. *J. Colloid interface Sci.* **1993**, *157*, 442.

8 (a) You, J. S.; Yu, X. Q.; Liu, K.; Tao, L., Xiang, Q. X.; Xie, R. G. *Tetrahedron: Asymmetry* **1999**, *10*, 243.
(b) Yan, J. M.; Xie, R. G.; Zhao, H. M. *Chin. J. Chem.* **1997**, *5*, 321.
(c) Luo, M. M.; Xie, R. G.; Yuan, D. Q.; Lu, W.; Xia, P. F.; Zhao, H. M. *Chinese J. Chem.* **1999**, *4*, 472.

9 Young, M. J.; Wahnon, D.; Hynes, R. C.; Chin, J. *J. Am. Chem. Soc.* **1995**, *117*, 9441.

10 Newcomb, M.; Timko, J. M.; Walba, D. M.; Cram, D. J. *J. Am. Chem. Soc.* **1977**, *99*, 6392.

11 Kimura, E.; Kuramoto, Y.; Koike, T.; Fujioka, H.; Kodama, M. *J. Org. Chem.* **1990**, *55*, 42.

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