Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(3):1520-1526

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Spectral analysis and catalytic activity in the hydrolysis of the phosphate ester by aza-crown ether cerium (III) complex

Bingying Jiang[1], Shulan Cai[2], Famei Feng[2] and Jiaqing Xie[1]*

[1]*College of Chemistry and Chemical Engineering, Chongqing University of Technology, Chongqing, China*
[2]*College of Chemistry & Pharmaceutical Engineering, Sichuan University of Science & Engineering, Zigong, Sichuan, China*

ABSTRACT

An aza-crown ether ligand, 4,7,13,16-tetraethoxycarbonylmethyl-1,10-dioxa-4,7,13,16-tetraaza-18-C-6, was synthesized and characterized in this work. The binary complex containing cerium ion (III) and the ligand was determined by fluorescence technique and then used as catalyst in the hydrolysis of bis(4-nitrophenyl) phosphate ester (BNPP). The experimental data indicate that the cerium (III) complex exhibited higher catalytic activity than that of other catalytic systems in BNPP hydrolysis. Based on the experimental data, the intramolecular hydrolytic cleavage mechanism was proposed and the correlative thermodynamic and kinetic data were determined by the kinetic model established in this work.

Key words: aza-crown ether, cerium (III) complex, catalysis, phosphate ester hydrolysis

---

INTRODUCTION

Phosphate ester plays numerous critical roles in biological systems, such as information storage and utilization (DNA/RNA), energy transduction (ATP), and cellular signaling communication [1]. Especially, phosphate esters specific hydrolysis plays an important role in the metabolic processes of living organism. Previous studies on the mechanism of phosphate ester hydrolysis have led to the development of a number of highly efficient artificial enzymes [2-4]. These studies have provided information about the fundamental role of metal ions in promoting hydrolytic reactions. Among these model compounds for among the lanthanides and it has been found to be particularly effective in promoting phosphatase, lanthanide ion and its complexes have attracted much attention as catalysts for phosphate ester hydrolysis [5,6] because of their extremely strong Lewis acidity, the conjunction of higher oxidation state and charge density, coordination number, and rapid ligand exchange rates [7, 8]. These characteristics make the lanthanide ions well-suited to be catalytic centers in the development of artificial enzymes [9]. Cerium is unique phosphodiester hydrolysis and DNA hydrolysis [10-12].

In a great number of biomimetic models of hydrolytic metalloenzymes, macrocyclic polyamine metal complexes employed in the catalyzing hydrolysis of phosphate esters have attracted considerable attention [13] due to the unique properties of macrocyclic polyamine, such as stabilizing metal ions with appropriate radii, introducing side-arms with different functional groups, which will exhibit different catalytic activities. Our research team has been working hard to develop the biomimetic models for metalloenzyme with high efficiency [14-18]. In the previous study, our research group also found that the two azamacrocyclic complexes can promote the hydrolytic cleavage of BNPP efficiently [19-20]. In order to better elucidate the role of lanthanide ions and further understand the mechanism of hydrolysis of phosphate esters catalyzed by the polyamine macrocyclic lanthanide (III) complex, a new system made of cerium ion (III) and an aza-crown ether ligand was constructed and used as catalyst or the phosphate ester hydrolysis in this work.

EXPERIMENTAL SECTION

Instrumentation and materials

Fluorescence spectrum was carried out on Cary Eclipse spectrofluorophotometer (Agilent Technologies Co. USA.). The elementary analysis was performed on a Carlo Erba 1106 elemental analyzer (Carlo-Erba Co. Italy). The pH of the solution was determined by using a Radiometer PHM 26 pH meter fitted with G202C glass and K4122 calomel electrodes (Shanghai photics apparatus Co. China). Melting points were determined on a Yanaco MP-500 micro-melting point apparatus (Yanaco-Mat Co. USA.) and uncorrected. UV-vis absorption spectrum and the kinetic studies were carried out by a TU-1900 UV-vis spectrophotometer equipped with a thermostatic cell holder (Beijing Purkinje General Instrument Co., Ltd., China).

The bis-(*p*-nitrophenyl) phosphate (BNPP) was purchased from Sigma Chemical Co. Other reagents used in the experiments, unless otherwise indicated, were of analytical grade, and was purchased from Chongqing chemical Co. The water used for kinetic experiments was doubly distilled water.

Synthesis of the ligand (L)

The synthesis method of the ligand, 4,7,13,16-tetraethoxycarbonylmethyl-1,10-dioxa-4,7,13,16- tetraaza-18-C-6 shown in figure 1, is based on the literature [21]. M. p. 58~59 ^{0}C. ^{1}H NMR ($CDCl_3$, TMS, 300 MHz) δ: 1.23~1.28 (t, 12H, 4×CH_3), 2.81~2.93 (q, 16H, 8×NCH_2), 3.42 (s, 8H, 4×NCH_2CO), 3.50~3.54 (t, 8H, 4×OCH2), 4.12~4.17 (q, 8H, 4×$COOCH_2$). Anal. calcd for $C_{28}H_{52}N_4O_{10}$: C, 55.61; H, 8.66; N, 9.26; Found: C, 55.62; H, 8.63; N, 9.96.

The preparation of the cerium (III) complex solution

The concentrated solution of cerium (III) nitrate and the ligand of 5×10^{-2} mol·dm^{-3} were prepared respectively in the doubly distilled water, and adjusted by adding base as necessary to pH = 7.0. The cerium (III) metal aza-macrocyclic complex solution of 5×10^{-3} mol·dm^{-3} was prepared by adding respectively the concentrated cerium nitrate and the concentrated ligand solution of 10 ml in the beaker under stirring 10 h and 100^{o}C, and then adding the mixture solution to the volumetric flask of 100 ml, and diluting with buffer solution of pH = 7.0. Stock solution of BNPP was prepared for a concentration of 2.0×10^{-2} mol·dm^{-3} in water.

Fluorescence spectra analysis

The fluorescence change of the cerium (III) ion was monitored with increase of the ligand in the solution of pH 7.0 under the condition of λ_{ex} of 254nm, λ_{em} of 265-500 nm, 650 V and 5 nm slit.

Kinetic method

Kinetic studies were carried out as follows: the reactions were initiated by injecting the desired volume (30 μL) of the substrate (BNPP) stock solution (2.0×10^{-2} mol·dm^{-3}) into the reaction cuvette. The kinetics of the substrate hydrolytic cleavage was monitored spectrophotometrically by the absorbance change of the product, p-nitrophenol, at 400 nm. To obtain reliable trends in observed reaction rate (k_{obsd}), measurements were performed within one day by using the same cells and stock solutions. The substrate catalytic hydrolysis is considered to be observed first-order reaction since the concentration of catalyst is at least 10 times higher than that of substrate BNPP. The observed first-order rate constants were obtained by the least square method with the equation $\ln(A_\infty-A_t) = k_{obsd}t + \ln(A_\infty-A_0)$. Where, A_0, A_t and A_∞ were initial absorbance, absorbance of moment t and final absorbance of the product of BNPP hydrolysis at 400nm, respectively. The data were obtained from the kinetic curves followed by up 95% or higher conversion of the substrate.

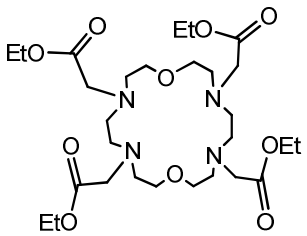


Fig. 1 The structure of the aza-macrocyclic ligand

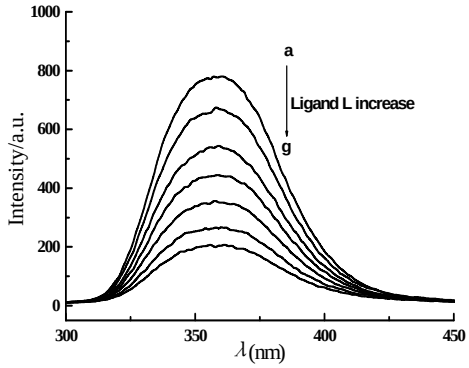


Fig. 2 Fluorescence spectra of the complex with increasing amounts of the ligand at pH 7.0 and T = 25°C
λ_{ex} = 254 nm, [Ce^{3+}] = 5×10^{-6} mol·dm^{-3}, [ligand] = 5.0×10^{-3} mol·dm^{-3}, ligand supplement 5 μL per scan, a~g: 0~30 μL

RESULTS AND DISCUSSION

Composition of the binary complex

Fluorescence method is becoming increasingly popular due to their easy use in solution as well as for their high sensitivity and selectivity. Fluorescent spectroscopy can also be used to study the interaction between metal ion and ligand. When the binding interaction between metal ion and ligand occurs, the fluorescence intensity of the system will enhance or weaken. This means the energy transfer occurs between the metal ion and ligand, which causes complexes fluorescence intensity to be weakened or enhanced [22]. In order to demonstrate the interaction between metal ion and ligand, the change of the fluorescence intensity of the system with addition of the ligand in the metal ion solution was analyzed in the present system. The fluorescent spectra was obtained at a fixed concentration of cerium ion and shown in figure 2. From this figure, it can be seen that the fluorescent intensity of the cerium ion decreases apparently upon the addition of the ligand, which indicates that there must have interactions between metal cerium ion and ligand.

The experimental results show that the fluorescence intensity of the metal cerium (III) ion reduced greatly with the addition of the ligand and the ligand is most probably a quencher agent of the fluorescent cerium (III). Therefore, when the concentration of ligand is fixed and is ten times higher than that of the cerium ion, the composition of the complex can be deduced from the following formula.

The reaction equation can be expressed as:
$nM + L = M_nL$

The chemical equilibrium constant can be expressed as:
$K = [M_nL] / [M]^n [L]$ (1)

According to eq.(1) give:
$\lg [M_nL] = n \lg[M] + \lg[L] + \lg K$ (2)

The fluorescence intensity should be proportional to the concentration of phosphor in the diluted solution, so we can obtain:
$F_0 = \alpha [M_0]$; $F = \alpha [M]$ (3)

According to the material balance, we can obtain:
$[M_nL] = ([M_0]-[M])/n$ (4)

Combination and rearrangement of eqs.(3) and (4) give:
$[M_nL] = (F_0 - F)/ \alpha n$ (5)

Combination of eqs. (2), (3) and (5) gives:
$\lg(F_0 - F) = n \lg F + \lg[L] + \lg K + \lg \alpha n - n \lg \alpha$ (6)

When L concentration of the solution is constant, the rearrangement of eq.(6) gives:
$\lg(F_0 - F) = n \lg F + \lg B$ (7)

Where, L is the ligand, M_nL is the binary complex, n is the combination number of cerium ion in the cerium (III)

complex, F_0 and F are the fluorescence of the cerium (III) ion in the aqueous solution and the ligand solution of the constant concentration, respectively.

According to equation (7), a plot of log (F_0−F) versus logF, which is shown as figure 3, is obtained by keeping the ligand concentration (5.0×10^{-5} mol·dm^{-3}) constant and changing the concentration of the metal ion. This figure gives straight line with a slope of 1.06, and is well fitted to eq. (2). This result indicates that the ligand and cerium (III) ion can form a stable complex according to the ratio of 1:1. Since the coordination number of the cerium (III) ion is usually 8 or 9, and the two molecules of the ligand can supply only 6 donor atoms to cerium (III) ion, the binary is containing at least two water molecules directly coordinated to the cerium (III) ion center. Therefore, the complex as catalyst can combine aqueous complex and then form the aqueous species $CeL(H_2O)_2$.

The survey of the catalytic activity of the different systems

The catalytic activity of the different systems is shown in Table 1. The experimental results indicate that the ligand shows no activity or BNPP hydrolysis. Cerium ion (III) solution shows low activity in our experimental conditions. It is interesting that the system composed of cerium ion (III) and the ligand exhibit reproducible and remarkably catalytic activity and stability in the phosphodiester cleavage. The observed first-order rate constant (k_{obsd}) of the BNPP catalytic cleavage is determined to be 4.52×10^{-4} s^{-1} in the solution system. Considering the spontaneous hydrolytic cleavage of BNPP at 25°C and pH 7, the catalyst of the solution system composed of cerium ion (III) and the ligand show an increase of the rate by 3 orders of magnitude compared to the cerium ion (III) salt in water, in which the BNPP reaction rate is about 10^4 times faster than that of BNPP spontaneous hydrolysis [23] under similar experimental conditions.

Table 1 k_{obsd} (s^{-1}) of BNPP hydrolysis catalyzed by the different systems

systems	L	H_2O	Ce^{3+}	Ce^{3+}+L
k_{obsd} (s^{-1})	No activity	1.2×10^{-11}	1.43×10^{-7}	4.52×10^{-4}

25°C, pH=7.0, [BNPP] = 2.0×10^{-5} mol·dm^{-3}, [L] = 1.0×10^{-3} mol·dm^{-3}
[Ce^{3+}] = 1.0×10^{-3} mol·dm^{-3}

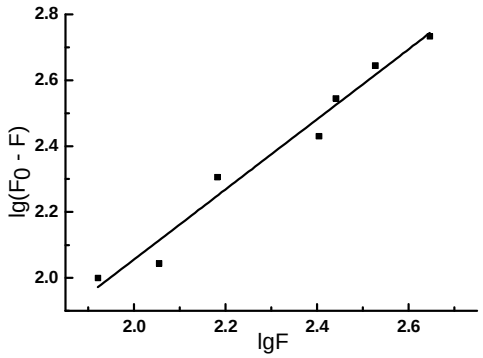


Fig. 3 Estimation of the composition of the complex made of the ligand and Ce^{3+}
λ_{ex}=254 nm, [ligand]= 5.0×10^{-5} mol·dm^{-3} at pH = 7.0 and T = 25°C.

BNPP binding assay

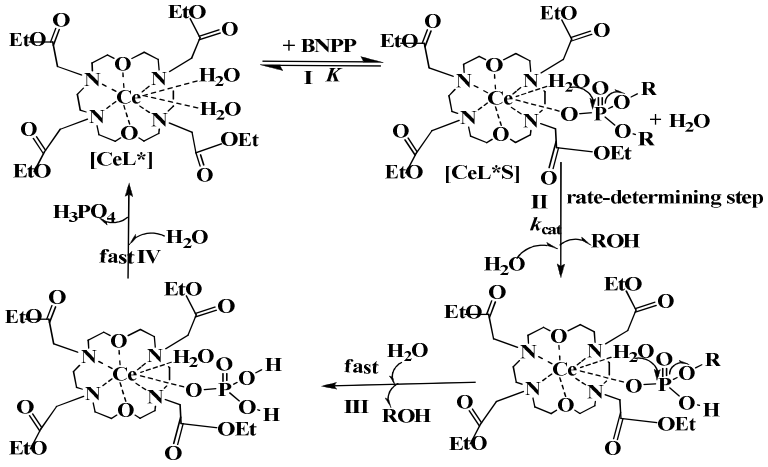
To clarify the interaction between the complex and BNPP in the solution, fluorescence studies were carried out in this work. The fluorescence spectra of the cerium complex were monitored both in the presence and absence of BNPP and the results shown in figure 4. This figure shows that the emission intensity of the complex decreases apparently with the increasing concentration of BNPP. The obvious decrease of fluorescence intensity of the complex by addition of BNPP indicates the strong binding occurs between the complex and BNPP molecule. The binding of the complex and BNPP can lead to the photoelectron transfer from BNPP to the excited state of the complex [24] and then the configuration change of the complex.

Mechanism of BNPP hydrolysis catalyzed by the cerium (III) complex

The result of BNPP binding assays proved the strong binding between BNPP molecule and metal cerium (III) complex. Our previous studies demonstrated that the binding occurs between metal ion of the complex and the phosphoryl oxygen atom of BNPP molecule [18-19]. To get more information about the reaction mechanism the hydrolytic rate of the p-nitrophenyl phosphate ester (NPP) catalyzed by the complex was also investigated. The experimental results show that the hydrolytic rate of the p-nitrophenyl phosphate ester (NPP) is ten times faster than that of BNPP in the $CeL(H_2O)_2$ system.

On the basis of the above experiment results, the mechanism of BNPP cleavage catalyzed by the title complex is

proposed in the following Scheme.



Scheme

This Scheme indicates that the phosphoryl oxygen of BNPP molecule bind to the metal ion of complex, which will facilitate the formation of the reaction intermediate containing BNPP molecule and the aqueous complex $CeL(H_2O)_2$ in the solution. In this process, the intermediate is stabilized and the negative charge of the substrate molecule is dispersed by the coordination of phosphoryl oxygen to Ce (III) ion, and the water molecule combined with the metal ion is activated by Ce (III) ion, which can promote an intramolecular nucleophilic reaction.

Kinetics of BNPP catalytic hydrolysis

Since the rate of BNPP spontaneous hydrolysis is much lower than that of catalytic hydrolysis, the products of spontaneous hydrolysis of BNPP can be neglected in kinetic equations. Furthermore, the step (II) in Scheme is the rate-determining step of the whole reaction. Hence, the rate equation of the catalytic reaction can be simplified as follows:

$$\text{Rate} = k_{obsd}[S]_T = k_{cat}[CeL^*S] \quad (8)$$

According to the mass balance, we have:

$$[S]_T = [S] + [CeL^*S] \quad (9)$$

Combination and rearrangement of eqs.(8) and (9) give:

$$k_{obsd} = \frac{k_{cat}\dfrac{[CeL^*S]}{[S]}}{1 + \dfrac{[CeL^*S]}{[S]}} \quad (10)$$

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

$$K = [CeL^*S] / [S][CeL^*] \quad (11)$$

where [CeL*] is the concentration of active species, combination of eqs.(10) and (11) leads to:

$$k_{obsd} = \frac{Kk_{cat}[CeL^*]}{1 + K[CeL^*]} \quad (12)$$

Rearranging eq.(12) can result in:

$$\frac{1}{k_{obsd}} = \frac{1}{k_{cat}} + \frac{1}{Kk_{cat}[CeL^*]} \quad (13)$$

In the above equations, [S] and $[S]_T$ are the free and the total concentration of the substrate BNPP, respectively; [CeR*] is the free concentration of the active species and can be substituted by the initial concentration; [CeL*S] is the concentration of the reactive intermediate formed by the substrate and the hydrated complex.

In order to prove the validity of the mechanism and mathematical model proposed above, the observed first order rate constants (k_{obsd}) of BNPP hydrolysis catalyzed by the catalytic system were obtained from the experiment with different concentrations of the complex. Based on the experiment data and equation (13), the relationship between $1/k_{obs}$ versus 1/[CeL*] was plotted in figure 5.

According to the intercept and slope of the straight line in figure 5 obtained by the linear fit of the least-square method, the k_{cat} of 4.50×10^{-1} s^{-1} and K of 5.11×10^{-4} $mol^{-1}\cdot dm^3$ can be evaluated with eq.(13). The curve in figure 5 show a good linear relationship between the variables, with $r^2 > 0.99$, which means that the reaction mechanism proposed in Scheme and the mathematical model established above is reasonable.

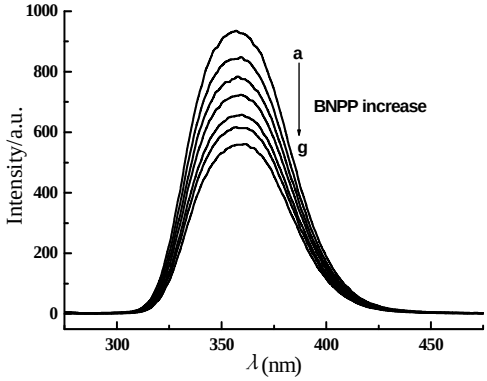


Fig. 4 Fluorescent spectra of the complex with increasing amounts of BNPP at pH 7.0 and T = 25°C. λ_{ex}=254 nm, [complex] = 5×10^{-5} mol·dm^{-3}, [BNPP] = 5.0×10^{-3} mol·dm^{-3}, BNPP supplement 5 μL per scan, a~g: 0~30 μL

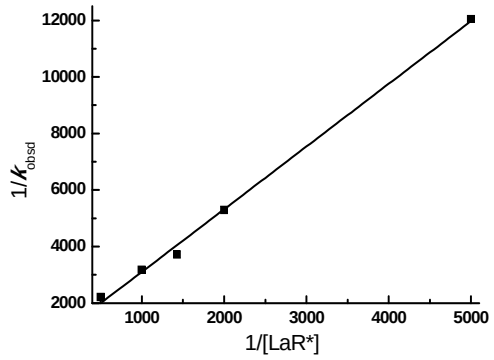


Fig. 5 Plot of $1/k_{obsd}$ versus 1/[BNPP] in the solution of the complex CeL
[complex]=2×10^{-5} mol·dm^{-3} at pH=7.0, T=25°C

CONCLUSION

In conclusion, the binary cerium (III) complex composed of cerium (III) ion and an aza-crown ether ligand in this work exhibit excellent catalytic activity in the hydrolysis of BNPP at neutral pH value and 25℃ and the reaction rate of BNPP hydrolysis increased by about 10^7 times compared with its spontaneous reaction rate. The interaction between cerium complex and BNPP molecule was investigated by the fluorescence technique and the decreased fluorescence intensity indicate the photoelectron transfer occurs between BNPP molecule and cerium complex. Based on the experimental data, the hydrolytic cleavage of BNPP catalyzed by title complex was carried through intramolecular pathway.

Acknowledgements
The authors gratefully acknowledge financial support from Chinese National Natural Science Foundation (21173274) and Major science and technology project of Zigong city (2013X03), and the Opening Project of Key Laboratory of Green Catalysis of Sichuan Institutes of High Education (LYJ1303).

REFERENCES

[1] MC Paul; S Erwin, *J. Am. Chem. Soc.*, **1999**, 121, 6125-6130.
[2] O Iranzo; AY Kovalecsky; JR Morow, *J. Am. Chem. Soc.*, **2003**, 125, 1988-1993.
[3] SS Tonde; AS Kumbhar; SB Padhye, *J. Inorg. Biochem.*, **2006**, 100, 51-57.

[4] DEC Ferreira; WBD Almeida; A Neves, *Phys. Chem. Chem. Phys.*, **2008**, 10, 7039-7046.
[5] H Katada; H Seino; Y Mizobe, J Sumaoka; M Komiyama, *J. Bio. Inorg. Chem.*, **2008**, 13, 249-255.
[6] MH Kuchma; C Komanski; J Colon, et al., *Nanomedicine: Nanotechnology, Biology and Medicine*, **2010**, 6, 738-744.
[7] AL Maldonado; AK Yatsimirsky, *Org. Biomol. Chem*., **2005**, 3, 2859-2867.
[8] PE Jurek; AM Jurek; AE Martell, *Inorg. Chem*., **2000,** 39, 1016-1020.
[9] SJ Franklin, *Curr. Opin. Chem. Bio*., **2001**, 5, 201–208.
[10] J Rammo; R Hettich; A Roigk; HJ Schneider, *Chem. Commun*., **1996**, 105-107.
[11] M Komiyana. *Chem. Commun.*, **1999**, 1443-1451.
[12] L Tjioet; T Joshit; CM Forsytht; B Moubaraki; KS Murray; J Brugger; B Graham; L Spiccia, *Inorg. Chem*., **2012**, 51(2), 939-953.
[13] F Mancin; PN Tecill, *New J. Chem.*, **2007**, *31*, 800-817.
[14] JQ Xie; BY Jiang; XM Kou; CW Hu; XC Zeng; YT Li, *Trans. Met. Chem*., **2003**, 28, 782-787.
[15] BY Jiang; Y Xiang; J Du; JQ Xie, et al., *Colloid Surf A*, **2004**, 235, 145-151.
[16] JQ Xie; FM Feng; SX Li; FA Liu, *J. Disper. Sci. Technol*., **2008**, 29, 1311-1318.
[17] JQ Xie; B Xie, FM Feng; LK Zou; JS Feng, *Prog. React. Kinet. Mech.*, **2009**, 34(3), 249-260.
[18] FM Feng; SL Cai; FA Liu; JQ Xie, *J. Chem. Pharm. Res.*, **2013**, 5(12):1389-1393.
[19] JQ Xie; C Li; M Wang; BY Jiang, *Int. J. Chem. Kinet.*, **2013**, 45, 397-403.
[20] JQ Xie; C Li; M Wang; BY Jiang, *Chem Papers*, **2013**, 67 (4), 365-371.
[21] Q Zhang; L Li; Y Liu, *Acta. Chimica. Sinica*., **2004**, 62(5), 514- 517.
[22] Y Huang; RD Yang; L Yan, *J. Chin. Rare Earth Soc*., **2002**, 20, 17-20.
[23] MJ Young; D Wahnon; RC Hynes; J Chin, *J. Am. Chem. Soc*., 1995, 117(37), 9441-9447.
[24] V Rajendiran; R Karthik; M Palaniandavar, et al., *Inorg. Chem.*, **2007**, 46(20), 8208-8221.