



Synthesis, Characterization and Antibacterial Activity of Ln(III) Complexes with Unsymmetrical Schiff Base Ligand

CAIFENG BI^{1,*}, JUN YANG¹, YUHUA FAN¹, SHUANGYU BI², DONGMEI ZHANG¹ and XIA ZHANG¹

¹Key Laboratory of Marine Chemistry Theory and Technology, Ministry of Education, College of Chemistry and Chemical Engineering, Ocean University of China, Qingdao 266100, P.R. China

²Academy for Advanced Interdisciplinary Studies, Peking University, Peking 100871, P.R. China

*Corresponding author: Fax: +86 532 66781932; Tel: +86 532 66781932; E-mail: bicaifeng301@163.com

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A new unsymmetrical tetradentate Schiff base (H₂L) was synthesized using 5-chloro-2-hydroxybenzophenone, *o*-phenylenediamine and *o*-vanillin. Three Ln(III) complexes of this Schiff base with the general composition [Ln(HL)(NO₃)₂(H₂O)]·2H₂O (Ln = Er, Pr, Gd), have been prepared and characterized by elemental analyses, IR spectra, UV spectra, ¹H NMR spectra, TG-DTG and molar conductance. The antibacterial activities of the ligand and its complexes are also studied. The antibacterial experiments indicate that the ligand and its complexes possess antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* and that the complexes have higher activity than that of the Schiff base.

Key Words: Unsymmetrical Schiff base, Ln(III) complex, Synthesis, Characterization, Antibacterial action.

INTRODUCTION

Symmetric tetradentate Schiff base complexes have been used extensively as macrocycle models¹, while unsymmetric complexes are very important in biological systems and in industrial catalysis^{2,3}. Recently, studies of some metal complexes with unsymmetrical Schiff bases derived from acetylacetone, ethylenediamine and salicylaldehyde have been reported^{4,5}. To continue the investigation in this area, a new unsymmetrical Schiff base ligand has been synthesized starting from 5-chloro-2-hydroxybenzophenone, *o*-phenylenediamine and *o*-vanillin. It is known that many complexes of rare earth metals possess antitumor, fungicidal and antibacterial activities^{6,7}, studies on the physico-chemical properties of rare earth complexes are appealing. The Schiff base synthesized in this paper is a chelating agent and the chelation of it with metal ions may act synergistically to increase its effect⁸. In the present work, we report the synthesis, characterization and antibacterial activities of the Schiff base ligand and its complexes with Er(III), Pr(III) and Gd(III). This paper offers a new method for preparing this kind of unsymmetrical Schiff base and its complexes, which is of significance in the fields of biology and material.

EXPERIMENTAL

All reagents used in this work were of analytical grade. 5-Chloro-2-hydroxybenzophenone was prepared by the reaction

of benzoyl chloride with 4-chlorophenol in the presence of anhydrous aluminum trichloride⁴. Hydrated Ln(III) nitrate was prepared by reaction of Ln(III) oxide with nitric acid.

Preparation of ligand

Mono-Schiff base (HR): *o*-Phenylenediamine (18.50 g, 171 mmol) dissolved in 150 mL anhydrous ethanol was mixed with piperidine (1.50 g, 17.6 mmol), triethyl orthoformate (25.2 g, 170 mmol) and 5-chloro-2-hydroxybenzophenone (39.80 g, 171 mmol) and refluxed for 6 h to give an orange-red precipitate. The precipitate was collected by filtration washed with ethanol and dried in a vacuum. Yield, 36.2 g (65.6 %); mp: 193-194 °C. Mass spectrum analysis of mono-Schiff base (HR) indicate that the molecular weight of HR is 322, which indicate that the reaction product of 5-chloro-2-hydroxybenzophenone with 1, 2-diaminobenzene is a mono-Schiff base. Anal. calcd for C₁₉H₁₅N₂OCl (m.w. 322.8): C, 70.69; H, 4.68; N, 8.68, found: C, 70.81; H, 4.61; N, 8.72 %.

Unsymmetrical Schiff base (H₂L): The prepared mono-Schiff base (4.00 g, 12.4 mmol) dissolved in 150 mL methanol was mixed with *o*-vanillin (1.89 g, 12.4 mmol) and refluxed for 4 h to give an orange-yellow precipitate. The precipitate was filtered, recrystallized with methanol and dried in a vacuum. Yield, 4.42 g (78 %); mp: 204-205. Mass spectrum analysis of H₂L indicate that the molecular weight of H₂L is 457, which indicate that the reaction product of mono-Schiff base HR with *o*-vanillin is an unsymmetrical di-Schiff base.

Preparation of complexes: The unsymmetrical Schiff base (1.83 g, 4.0 mmol) dissolved in 60 mL butyl alcohol was mixed with the lanthanide nitrate (4.0 mmol) dissolved in 20 mL methanol and refluxed for 4 h to give a precipitate. The precipitate was filtered, recrystallized from methanol and dried in a vacuum. The purity of the complexes was higher than 99 %.

Elemental analyses were carried out with a model 2400 Perkin-Elmer analyzer. The metal content was determined gravimetrically. The ultraviolet spectra were recorded on a Shimadzu UV-3000 spectrophotometer in DMSO. The molar conductance was measured with a Shanghai DDS-11A conductivity meter. Infrared spectra of the ligand and complexes were recorded in KBr pellets using a Bio-Rad FTS 165 spectrophotometer. Thermogravimetric measurements were made using a Perkin-Elmer TGA7 instrument. The heating rate was programmed to be 10 °C/min with a protecting stream of N₂ flowing at a rate of 40 mL/min. The mass spectrogram of the ligand was recorded on a Finnegan MAT-212 mass spectrometer. ¹H NMR spectra were recorded in DMSO-D₆ as the solvent at 600 MHz with a JNM ECP-600 spectrometer using tetramethylsilane (TMS) as an internal reference.

RESULTS AND DISCUSSION

The synthesis reactions of the ligand are shown in Fig. 1.

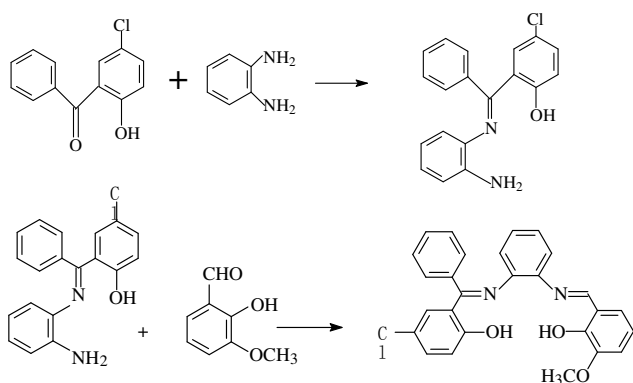
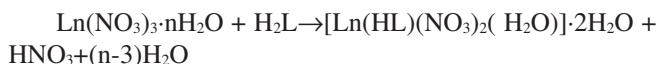


Fig.1. Preparation of the ligand

The synthesis reaction of the complexes may be represented as shown below.



The results of the elemental analyses and molar conductance data are shown in Table-1. The molar conductance values of the complexes measured in DMSO in 10⁻³ mol L⁻¹ solution fall in the range 9.98-13.2 S cm² mol⁻¹ indicating their non-electrolytic nature⁹. The complexes are stable in air and soluble in DMF and DMSO; however they are insoluble in benzene, acetone, diethyl ether or water.

IR Spectra: The infrared spectra of the ligand and its complexes are shown in Table-2. The shift of ν(C=N) from 1631.5 cm⁻¹ in the ligand to very strong absorption bands in the region 1611-1624 cm⁻¹ in the complexes, suggests the formation of a C=N-Ln band system. The vibration ν(C-O) of H₂L occurs at 1192 cm⁻¹. The shift to lower frequency by about 8 cm⁻¹ in the lanthanide(III) complexes indicates the coordination of oxygen to metal ions. In the spectra of the complexes the occurrence of bands at 467-437 cm⁻¹ (Ln-O) and 581-544 cm⁻¹ (Ln-N) provides evidence for the bonding of oxygen and nitrogen to the central metal ion¹⁰. In the spectra of the complexes, three additional bands, which are not present in the spectrum of the ligand, were observed. Of these, the band around 1039 cm⁻¹ is assigned to the ν₂ mode of the nitrate group. The bands in the region 1506-1502 cm⁻¹ and 1236-1228 cm⁻¹ in all the complexes are the two split bands of ν₄ and ν₁, respectively, of the coordinated nitrate group. The magnitude of (ν₄-ν₁) is more than 180 cm⁻¹ for the complexes, which indicates that the nitrate group is coordinated to the metal ion in a bidentate fashion¹¹. The spectra of the complexes exhibit a broad band at 3115-3124 cm⁻¹, which is attributed to ν(O-H) of the water molecules of crystallization⁹.

Electronic spectra: The electronic spectral data are shown in Table-3. The electronic spectra of the complexes in DMSO exhibit the same two spectral bands in the regions at 264-267 nm and 352-354 nm, which are also observed in the spectrum of the ligand. Compared with the electronic spectrum of the ligand, there is a shift to lower frequency, which further testifies to the formation of complexes.

¹H NMR Spectra: The ¹H NMR spectra of the ligand and complex [La(HL)(NO₃)₂(H₂O)]·2H₂O were recorded in DMSO-D₆. In the spectrum of the ligand the phenolic OH proton appears at 14.18 ppm. This signal shifts to 10.21, 12.95 ppm in the complex spectrum, which indicates that the coordination of phenolic oxygen to metal ions. The peak at 9.39 ppm in the ligand can be assigned to CH=N proton. It shifts to 8.70 ppm in the spectrum of the complex, which confirms the

TABLE-1
ANALYTICAL AND PHYSICAL DATA OF THE LIGAND AND ITS COMPLEXES

Compounds	Empirical Formula	Formula Weight	Yields (%)	Colour	D.P. ^a (°C)	Anal. Found (Calcd.) %				Molar conductance S Mol ⁻¹ cm ²
						C	H	N	M	
H ₂ L	C ₂₇ H ₂₁ N ₂ O ₃ Cl	456.9	78	Orange	204 ^b	71.27	4.67	6.16		
[Er(HL)(NO ₃) ₂ (H ₂ O)]·2H ₂ O	C ₂₇ H ₂₆ N ₄ O ₁₂ ClEr	801.3	77	Yellow	275	40.75	3.24	6.97	20.21	13.2
[Pr(HL)(NO ₃) ₂ (H ₂ O)]·2H ₂ O	C ₂₇ H ₂₆ N ₄ O ₁₂ ClPr	774.9	74	Yellow	270	41.88	3.33	7.21	18.39	10.8
[Gd(HL)(NO ₃) ₂ (H ₂ O)]·2H ₂ O	C ₂₇ H ₂₆ N ₄ O ₁₂ ClGd	791.3	71	Yellow	279	41.97	3.37	7.10	18.94	9.98
						(40.98)	(3.31)	(7.08)	(19.88)	

^aDecomposition point, ^bMelting point.

TABLE-2
 MAIN IR BANDS OF LIGAND AND COMPLEXES (cm⁻¹)

Compounds	$\nu(\text{O-H})$	$\nu(\text{C=N})$	$\nu(\text{C-O})$	$\nu(\text{N-M}), \nu(\text{O-M})$	$\nu(\text{NO}_3^-)$
H ₂ L	3149.2s	1631.5s	1192.1m		
[Er(HL)(NO ₃) ₂ (H ₂ O)]·2H ₂ O	3124.1s	1616.1s	1181.7m	543.7w 467.3w	1506.4s 1232.5m 1033.6m
[Pr(HL)(NO ₃) ₂ (H ₂ O)]·2H ₂ O	3115.0s	1624.3s	1186.6m	544.1w 437.2w	1502.6s 1236.0m 1046.5m
[Gd(HL)(NO ₃) ₂ (H ₂ O)]·2H ₂ O	33119.0s	1611.3s	1184.9m	581.3w 437.6w	1506.2s 1228.0m 1039.2m

 TABLE-3
 UV SPECTRA FOR LIGAND AND COMPLEXES

Compounds	$\lambda_{\text{max1}}(\text{nm})$	$\epsilon_1 \times 10^{-4} (\text{L mol}^{-1} \text{cm}^{-1})$	$\lambda_{\text{max2}}(\text{nm})$	$\epsilon_2 \times 10^{-4} (\text{L mol}^{-1} \text{cm}^{-1})$
H ₂ L	260	1.42	348	0.518
[Er(HL)(NO ₃) ₂ (H ₂ O)]·2H ₂ O	266	1.46	354	0.476
[Pr(HL)(NO ₃) ₂ (H ₂ O)]·2H ₂ O	267	1.62	352	0.439
[Gd(HL)(NO ₃) ₂ (H ₂ O)]·2H ₂ O	264	1.53	352	0.345

coordination of azomethine group to metal ion. In the spectrum of complex the multi signals within the 7.38-7.55 ppm range are assigned to aromatic H protons. In the spectrum of the ligand the -OCH₃ proton appears at 3.38 ppm and it appears at 3.33 ppm in the complex spectrum, which indicates the -OCH₃ is not band to metal ion.

Thermal decomposition studies: The DTG-TG curves of the Pr(III) complex were determined in a stream of N₂ at a flowing rate of 40 mL/min. The complex decomposes in four steps. The first weight loss stage has a decomposition temperature range of 60-125 °C, with a weight loss of 4.82 %, which corresponds to the loss of two molecules of water (calcd. 4.65 %). The fact that the water molecule was lost at a low temperature suggests that the water is crystal water. The second weight loss stage has a decomposition temperature range of 142-273 °C, with a weight loss of 2.54 %, corresponding to the loss of one molecule of water (calcd. 2.33 %). The fact that the water molecule was lost at a higher temperature suggests that the water is coordinated with the metal ions¹². The third weight loss stage showed a continuous weight loss between 273 and 472 °C, with a weight loss of 39.13 %, which corresponds to the loss of the mon-Schiff base (calcd. 39.45 %). The fourth stage showed a continuous weight loss between 472 and 830 °C and 22.35 % of the original sample remained. With its calculated weight percentage of 21.97 %, Pr₆O₁₁ is the final product.

Antibacterial activity: *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* were cultured by inoculating in beef broth (which was sterilized by autoclaving at 121 °C for 30 min) and incubating at 37 °C for 24 h. Agar culture medium, which was sterilized by autoclaving at 121 °C for 20 min, was prepared by dissolving peptone, beef broth, agar and sodium chloride in distilled water and adjusted pH to 7.0~7.2. Cultured *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* were added to the warm nutrient agar, which was poured into plates that were allowed to set in the refrigerator for at least 6 h. The agar plates which were incubated at 37 °C overnight allowing the bacterium to grow where possible. The compounds solutions (0.5 mg/mL) were obtained by dissolving the ligand or complexes in aseptically normal saline (w = 0.009) and DMSO in the proportion of 4:1 (v/v).

Their minimal inhibition concentration (MIC) to the growth of bacterium has been determined using agar dilution¹³. The ligand or complexes solutions of various concentrations

were added to agar plates and the plates not existing compounds were tested as positive control. A 2 × 10⁴ mL bacterium suspension was also spread onto agar plates. The plates were then incubated for 24 h at 37 °C for bacteria. The minimal concentrations of the compounds solutions at, which the bacteria did not grow in the agar plates are their minimal inhibition concentrations (MIC).

The minimal inhibition concentrations of various compounds are shown in Table-4. From the data it is observed that the present Schiff base and its lanthanide complexes have antibacterial activity to the tested bacterium and the complex has the higher activity than the ligand. Gd(III) complex shows a higher activity than the other complexes. The structure activity relation about their bioactivities will be studied further.

 TABLE-4
 THE MINIMAL INHIBITION CONCENTRATION OF THE LIGAND AND ITS COMPLEXES (MIC × 10⁵ g mL⁻¹)

Compound	<i>E. coli</i>	<i>S. aureus</i>	<i>B. subtilis</i>
H ₂ L	12.5	12.5	6.25
[Er(HL)(NO ₃) ₂ (H ₂ O)]·2H ₂ O	3.13	3.13	3.13
[Pr(HL)(NO ₃) ₂ (H ₂ O)]·2H ₂ O	6.25	6.25	3.13
[Gd(HL)(NO ₃) ₂ (H ₂ O)]·2H ₂ O	1.56	3.13	1.56

Conclusion

The results presented here clearly indicate that Er(III), Pr(III) and Gd(III) can form stable solid complexes with H₂L. The compositions of the complex is confirmed to be [Ln(HL)(NO₃)₂(H₂O)]·2H₂O. The structure proposed for the complexes is shown in Fig. 2. The complexes have higher antibacterial activity than that of the ligand.

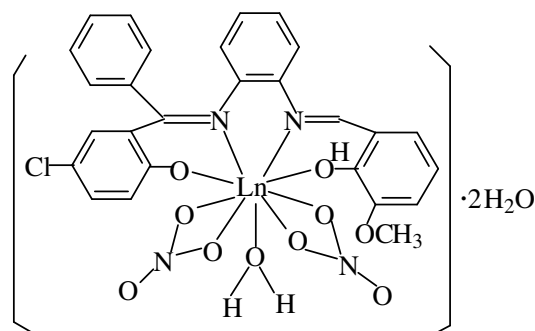


Fig. 2. Suggested structure of the complexes (Ln = Er, Pr, Gd)

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