Inorg. Chem. **1996,** *35,* 6513−6519 **6513**

Complexes of Zn^{2+}, Cd^{2+}, and Hg^{2+} with 2-(α-Hydroxybenzyl)thiamine Monophosphate Chloride

Katerina Dodi,[†] Ioannis P. Gerothanassis,[†] Nick Hadjiliadis,*,[†] Andre Schreiber,[‡]
Robert Bau,[‡] Ian S. Butler,[§] and Patrick J. Barrie[||,⊥]

Department of Chemistry, University of Ioannina, Ioannina 45-110, Greece, Department of Chemistry,
University of Southern California, Los Angeles, California 90089-1062, Department of Chemistry,
McGill University, 801 Sherbrooke Street West, Montreal, PQ, H3A 2K6 Canada, and Department of
Chemistry, University College London, 20 Gordon Street, London WC1H OAJ, U.K.

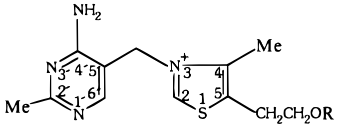
Received June 15, 1995[⊗]

The binding sites of Zn^{2+}, Cd^{2+}, and Hg^{2+} in complexes with 2-(α-hydroxybenzyl)thiamine monophosphate chloride, $(LH)^+Cl^-$, have been investigated in the solid state [2-(α-hydroxybenzyl)thiamin monophosphate chloride monoprotonated at the phosphate group and protonated at N_1' is denoted as $(LH)^+Cl^-$; therefore, the ligand monoprotonated at the phosphate group and deprotonated at N_1' is L]. Complexes of formulae $MLCl_2$, $M(LH)Cl_3$, and $(MCl_4)^{2-}(LH)_2{}^+$ ($M = Zn^{2+}$, Cd^{2+}, and Hg^{2+}) were isolated in aqueous and methanolic solutions, depending on pH. The crystal structure of the complex of formula HgL_2Cl_2 was solved, together with that of the free ligand $(LH)^+Cl^-$, by X-ray crystallography. HgL_2Cl_2 crystallizes in *C*2/*c*, with $a = 32.968(6)$ Å, $b = 7.477(2)$ Å, $c = 21.471(4)$ Å, $\beta = 118.19(1)°$, $V = 4665(2)$ Å^3, and $Z = 4$. $(LH)^+Cl^-$ crystallizes in *Cc*, with $a = 10.951(3)$ Å, $b = 17.579(4)$ Å, $c = 13.373(3)$ Å, $\beta = 105.36(2)°$, $V = 2482.4(10)$ Å^3, and $Z = 4$. Mercury(II) binds to the N(1′) of the pyrimidine ring. Both ligands are in the *S* conformation [$\Phi_T = -98.1(9)°$ and $\Phi_P = 176.1(10)°$ for HgL_2Cl_2 and $\Phi_T = 104.1(5)°$ and $\Phi_P = 171.9(6)°$ for $(LH)^+Cl^-$]. ^{31}P and ^{13}C NMR spectra, together with vibrational spectra (IR/Raman), are used to deduce the binding sites of the metal and the protonation states of the ligand at various pH values. It is found that solid-state ^{31}P NMR spectroscopy is particularly useful in characterizing these complexes as the ^{31}P shielding tensors are sensitive to the state of the phosphate group. On the other hand, the ^{31}P NMR spectra indicate that direct bonding between Zn^{2+} and Cd^{2+} to the phosphate can occur under certain preparation conditions. Solid-state ^{13}C NMR and vibrational (IR/Raman) spectroscopic results are also in agreement with the other techniques.

Introduction

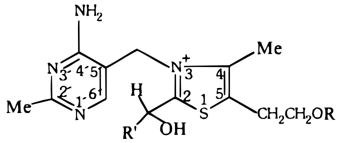
The pyrophosphate derivative of thiamine (vitamin B_1) is well-known to require the presence of divalent metal ions, such as Mg^{2+} and Ca^{2+} *in vivo* and Co^{2+}, Zn^{2+}, Mn^{2+}, Cd^{2+} etc. *in vitro*, to act as a cofactor for enzymes involved in catalyzing reactions such as the decarboxylation of α-keto acids or the transfer of aldehyde and acyl groups (decarboxylase, transketolase, etc.).[1,2] It has been suggested that the metal acts as a bridge between the apoenzyme and the thiamine cofactor.[2] Because of this, there have been a number of studies attempting to establish the role of the divalent cation by determining the metal-binding site to the thiamine derivative.[3] However, in many cases ionic salt complexes form with a metal-containing anion balancing the positive charge located on the thiazolium ring.[4,5] In those cases where direct bonding to thiamine is observed, the metal normally forms a covalent bond to the N(1′) site of the pyrimidine ring;[6,7] however, complexes with coordination to the hydroxyethyl oxygen of thiamine[7,8] or the pyrophosphate group of the pyrophosphate derivative are also known.[9] So-called "active aldehyde" derivatives (**1**, **2**) are known intermediates in the enzymatic process,[10] and it has been found that Zn^{2+}, Cd^{2+}, and Hg^{2+} bind to the N(1′) site of the active aldehyde derivatives of thiamine (containing no phosphate group) in both the solid state and solution.[11,12]

1



R=H Thiamine
$R=HPO_3{}^-$ Thiamine monophosphate
$R=H_2P_2O_6{}^-$ Thiamine pyrophosphate

2



R′=Ph 2-(α-Hydroxybenzyl)thiamine

In this paper, we report solid-state NMR and vibrational spectroscopy results on Zn^{2+}, Cd^{2+}, and Hg^{2+} complexes of the active aldehyde derivative 2-(α-hydroxybenzyl)thiamine monophosphate chloride [denoted $(LH)^+Cl^-$ throughout this paper for convenience] prepared at pH 3.5 and 5.5−6.0.

---

* Corresponding author.
[†] University of Ioannina.
[‡] University of Southern California.
[§] McGill University.
[||] University College London.
[⊥] Current address: Dept. of Chemical Engineering, University of Cambridge, Pembroke Street, Cambridge CB2 3RA, UK.
[⊗] Abstract published in *Advance ACS Abstracts,* September 15, 1996.

(1) Scrutton, M. C. *Inorg. Biochem.* **1973,** *1,* 3.
(2) Schellenberger, A. *Angew. Chem., Int. Ed. Engl.* **1967,** *6,* 1024.
(3) Louloudi, M.; Hadjiliadis, N. *Coord. Chem. Rev.* **1994,** *135,* 429.
(4) Richardson, M. F.; Franklin, K.; Thomson, D. M. *J. Am. Chem. Soc.* **1975,** *97,* 3204.
(5) Aoki, K.; Tokuno,T.; Takagi, K.;Hirose, Y.; Suh, I. H.; Adeyemo A. O.; Williams, G. N. *Inorg. Chim. Acta* **1993,** *210,* 17.
(6) Cramer, R. E.; Maynard, R. B.; Ibers, J. A. *J. Am. Chem. Soc.* **1981,** *103,* 76.
(7) Aoki, K.; Yamazaki, H.; Adeyemo, A. *Inorg. Chim. Acta* **1991,** *180,* 117.
(8) Hu, N. H. *Inorg. Chim. Acta* **1991,** *186,* 209.
(9) Aoki, K.; Yamazaki, H. *J. Am. Chem. Soc.* **1980,** *102,* 6878.
(10) Breslow, R. *J. Am. Chem. Soc.* **1958,** *80,* 3719.

S0020-1669(95)00758-0 CCC: $12.00 © 1996 American Chemical Society

Preparations at lower pH values have a tendency to form ionic salt complexes of the type $[LH^+]_2[MCl_4]^{2-}$ with no direct binding between metal and thiamine. The spectroscopic results provide indirect information on the metal-binding site. In particular, the ^{31}P solid-state NMR results enable the principal components of the ^{31}P shielding tensor to be obtained, which sheds light on the state of the phosphate group and, hence, on the mode of metal binding. The spectroscopic studies are complemented by single-crystal X-ray structure determinations of the uncomplexed molecule $(LH)^+Cl^-$ prepared at pH 3.5 and the mercury complex HgL_2Cl_2. Unfortunately we have not been able to prepare crystals of Zn^{2+} or Cd^{2+} complexes with L of sufficient quality for X-ray structure determination. However, the agreement between the conclusions drawn from the spectroscopic data and the crystal structure of the Hg^{2+} complex means that we can be fairly confident about inferences based solely on spectroscopic data for the other complexes. It is found for the first time that direct binding of the metal to the monophosphate group can occur for the Zn^{2+} and Cd^{2+} complexes for preparations at certain pH values.

Experimental Section

Materials. Thiamine monophosphate chloride was purchased from Sigma Chemical Co. and used without further purification. $ZnCl_2$, $CdCl_2$, and $HgCl_2$ and all other chemicals used were from Aldrich AG.

Preparation of the Compounds. 1. Preparation of the Ligand 2-(α-Hydroxybenzyl)thiamine Monophosphate Chloride $(LH)^+Cl^-$. 2-(α-Hydroxybenzyl)thiamine monophosphate chloride was prepared by employing a method analogous to that used for the preparation of 2-(α-hydroxybenzyl)thiamine[11] but using thiamine monophosphate chloride as the starting material.

Various forms of the ligand were prepared by dissolving 100 mg in aqueous solutions at pH 6, 3.5, and 1 by using 0.1 N solutions of KOH or HCl. The solutions were then evaporated to dryness. To help evaporation, small quantities of ethanol were added to the aqueous solution of the ligand at pH 1. Solution proton undecoupled ^{31}P NMR spectra of the ligand at pH 1 indicate partial hydrolysis upon heating, increasing with time (absence of the characteristic $^3J_{P-H}$ coupling constant). This reaction was not investigated further at present. They were dried first at room temperature and then at 55−60 °C *in vacuo*.

Crystals of $(LH)^+Cl^-$ were obtained by dissolving a small amount of product in water (pH 3.5) in a small test tube and putting it in a stoppered erlenmeyer flask containing ethanol. After diffusion of the ethanol into the water solution at room temperature over several days, crystals of $(LH)^+Cl^-$ appeared in the test tube. The crystal structure indicated that three molecules of water were also present per thiamine unit cell.

2. Preparation of the $MLCl_2$ Complexes ($M = Zn^{2+}$, Cd^{2+}). These complexes were prepared by mixing equimolar (1 mmol) amounts of $(LH)^+Cl^-$ and the metal chlorides ($ZnCl_2$, $CdCl_2$) in methanolic solutions (20−30 mL). The pH meter was calibrated with aqueous buffer solutions. Then 0.1 N KOH solution was added to both methanolic solutions of the ligand and the metal chloride up to pH 5.5−6 (pH meter reading) and filtered from any insoluble material prior to mixing. The resulting white precipitates, after the mixture was stirred for about 2 h at room temperature, were filtered and washed with small quantities of methanol and ether. They were then dried *in vacuo* in the presence of $CaCl_2$ first at room temperature followed by heating to 50−60 °C. Elemental analysis suggested that three water molecules were present per thiamine unit for both $ZnLCl_2$ and $CdLCl_2$ (yields ∼ 50%).

3. Preparation of the $M(LH)Cl_3$ Complexes ($M = Zn^{2+}$, Cd^{2+}). These were prepared as in the previous case by adjusting the pH to about 3.5. The best agreement with elemental analysis was for a single water molecule of crystallization to be present per thiamine unit (yields ∼ 50%).

4. Preparation of the $(LH)_2{}^+(MCl_4)^{2-}$ Complexes ($M = Zn^{2+}$, Cd^{2+}, Hg^{2+}). These were prepared by following the procedure used for the $MLCl_2$ complexes by adjusting the pH of both the ligand and the metal chloride to about 1−1.5 using 0.1 N HCl solution (yields ∼ 50%).

5. Preparation of HgL_2Cl_2. Equimolar amounts (1 mmol) of $HgCl_2$ and the ligand $(LH)^+Cl^-$ were mixed in aqueous solutions (20−30 mL) after adjusting the pH to 5.5−6, using 0.1 N KOH solution. After the mixture was stirred for 24 h at room temperature, the resulting precipitate was filtered and washed with small quantities of water, methanol, and ether. It was dried first at room temperature and then at 55−60 °C *in vacuo*, in the presence of $CaCl_2$. The crystal structure indicated that one molecule of water was present per thiamine unit (yield ∼ 90% based on $HgCl_2$).

6. Preparation of HgL_2Cl_2 Crystals. An aqueous solution (10 mL 10^{-2} M) of the ligand $(LH)^+Cl^-$ and 10 mL of a 10^{-2} M solution of $HgCl_2$ in a mixture of ethanol and hexane (3:2) were mixed in a test tube. The tube was left in the refrigerator, and after about 10 days crystals of the complex appeared at the interface of hexane and water.

7. Deuteration Experiments. The deuterated ligands at various pH's and the complexes were prepared by dissolving small amounts (30−40 mg) in about 1 mL of D_2O, followed by lyophilization. The deuterated HgL_2Cl_2 complex was prepared by carrying out the preparation in D_2O solution on a smaller scale (0.1 mmol).

Methods. 1. Crystal Structures. (a) The Ligand 2-(α-Hydroxybenzyl)thiamine Monophosphate Chloride Trihydrate ($(LH)^+Cl^-\cdot 3H_2O$). A crystal of size 0.2 × 0.2 × 0.6 mm was mounted in a glass capillary. Intensity data were collected with ω-scans, using a variable scan speed (6.00−60.0° min^{-1} in ω) and a scan width of 2.0°. A Siemens P_4 diffractometer with graphite-monochromated Mo Kα radiation was used for preliminary examination and data collection. The lattice parameters were determined from a least-squares fit of the setting angles of 45 reflections up to $2\theta_{max}$ of 25°. Three standard reflections were recorded at 1000 reflection intervals, and random deviations were detected during data collection. A total of 4159 reflections was measured with $4.0° < 2\theta < 45.0°$, having indices of $-7 < h < 11$, $-9 < k < 18$, and $-14 < l < 12$. The data were reduced and corrected for Lorentz polarization effects and averaged ($R_{int} = 0.040$) to 1832 unique reflections, 1752 of which had $F > 4.0\sigma(F)$. The space group was determined to be *Cc*. The structure was solved via direct methods. It was refined (on *F*) by using full-matrix least squares with anisotropic displacement parameters of all non-hydrogen atoms and a common isotropic displacement parameter for the H-atoms, which were placed in geometrically calculated positions. A total of 307 parameters was refined to $R = 0.035$, $wR = 0.042$, and $(\Delta/\sigma)_{max} = 0.091$. The programs used were SHELX 76 (Sheldrick, 1976) and TEXSAN (Molecular Structure Corporation Version 1.6, 1993).

(b) The Complex Bis[2-(α-hydroxybenzyl)thiamine]mercury(II) Dichloride (HgL_2Cl_2). A crystal of size 0.2 × 0.4 × 0.5 mm was mounted in a glass capillary. Intensity data were collected with ω-scans, using a variable scan speed (6.0−60.0° min^{-1} in ω) and a scan width of 2.0°. A Simens P_4/RA diffractometer with graphite-monochromated Cu Kα radiation was used for preliminary examination and data collection. The lattice parameters were determined from a least-squares fit of 55 reflections up to a maximum 2θ of 56°. Four standard reflections were recorded every 1000 reflections, and only random deviations were detected during data collection. A total of 3396 reflections with $4.0° < 2\theta < 102.5°$, $-33 < h < 32$, $-1 < k < 7$, and $-21 < l < 19$ was measured. The data were reduced and corrected for Lorenz polarization effects and averaged ($R_{int} = 0.041$) to 2508 unique reflections, 2149 of which had $F > 4.0\sigma(F)$. The space group was determined to be *C*2/*c*. The structure was solved via direct methods. It was refined (on *F*) by using full-matrix least squares with anisotropic displacement parameters for all non-hydrogen atoms and a common isotropic displacement parameter for the H-atoms, which were placed in geometrically calculated positions. A total of 289 parameters was refined to $R = 0.049$, $wR = 0.058$, and $(\Delta/\sigma)_{max} = 0.009$. The programs used were SHELX 76 (Sheldrick, 1976) and TEXSAN (Molecular Structure Corporation, Version 1.6, 1993).

2. ^{13}C and ^{31}P CP/MAS NMR Spectra. Solid-state ^{31}P NMR spectra were acquired by using cross-polarization (CP), magic-angle spinning (MAS), and high-power proton decoupling at 121.5 MHz on

---

(11) Louloudi, M.; Hadjiliadis, N.; Feng, J. A.; Sukumar, S.; Bau, R. *J. Am. Chem. Soc.* **1990,** *112,* 7233.

(12) Louloudi, M.; Hadjiliadis, N.; Butler, I. S. *J. Chem. Soc., Dalton Trans.* **1992,** 1401.

Table 1. Summary of Crystal Data and Refinement Results for $(LH)^+Cl^-\cdot 3H_2O$ and $HgL_2Cl_2\cdot H_2O$

	$C_{19}H_{30}N_4O_8PSCl$	$C_{38}H_{46}HgCl_2N_8O_{12}P_2S_2$
space group	Cc	*C*2/*c*
a, Å	10.951(3)	32.968(6)
b, Å	17.579(4)	7.477(2)
c, Å	13.373(3)	21.471(4)
β, deg	105.36(2)	118.19(1)
V, Å^3	2482.4 (10)	4665(2)
molecules/unit cell	4	4
formula weight	537.9	1204.4
cryst dimens, mm	0.2 × 0.2 × 0.7	0.2 × 0.4 × 0.5
calcd density, g cm^{-3}	1.439	1.715
absorption coefficient (μ), mm^{-1}	0.353	9.04
data collectionn wavelength, Å	0.7107	1.5418
$2\theta_{max}$, deg	45	102.5
total no. of reflections measd	4159	3396
no. of independent reflections	1832	2508
no. of reflections observed ($F > 4.0\sigma(F)$)	1752	2149
no. of variable parameters	307	289
final agreement factors	$R = 0.035$ $wR = 0.042$	$R = 0.049$ $wR = 0.058$
GOF	1.101	1.209
scan type	ω	ω
scan speed, deg/min	variable 6.00−60.00 in ω	variable 6.00−60.00 in ω
scan range (ω), deg	2.00	2.00

a Brucker MSL-300 multinuclear spectrometer. Spinning rates of 1.8−10 kHz were employed. The spectra obtained at the slow spinning rates enabled the principal components of the ^{31}P shielding tensor to be obtained by simulation of the spinning sideband intensities using an iterative fit procedure based on the method of Herzfeld and Berger.[13,14] The contact time used was 3 ms, and recycle delays were 2.5−15 s. Chemical shifts are relative to extermal 85% phosphoric acid. Following the proposal of Mason,[15] the principal components are given using the convention $\delta_{11} > \delta_{22} > \delta_{33}$, together with the span, Ω, defined as $\delta_{11} - \delta_{33}$, and the skew, κ, defined as $3(\delta_{22} - \delta_{iso})/\Omega$. To enable easy comparison with existing literature, values of the anisotropy, $\Delta\sigma$, and the asymmetry parameter, η, are also quoted: when $\delta_{11} - \delta_{22} < \delta_{22} - \delta_{33}$, $\Delta\sigma = 0.5(\delta_{11} + \delta_{22}) - \delta_{33}$ and $\eta = (\delta_{22} - \delta_{11})/(\delta_{33} - \delta_{iso})$; when $\delta_{11} - \delta_{22} > \delta_{22} - \delta_{33}$, $\Delta\sigma = 0.5(\delta_{33} + \delta_{22}) - \delta_{11}$ and $\eta = (\delta_{22} - \delta_{33})/(\delta_{11} - \delta_{iso})$.[16] ^{13}C CP/MAS NMR spectra were obtained by using the same conditions as described previously, with chemical shifts quoted relative to TMS.[12]

3. Vibrational Spectroscopy. The IR spectra were obtained on a Perkin-Elmer 783 instrument using either KBr disks or Nujol mulls. The Raman spectra were obtained by using a Spex 1403 spectrometer with a Spectra-Physics Model 2020 argon ion laser. Excitations were performed with a wavelength of 514.5 mm.

4. Elemental Analyses. The elemental analyses of all forms of ligands and the complexes were carried out in our laboratory by using an EA-1108 Carlo Erba analyzer.

Results and Discussion

Crystal Structures. Details of the crystal structure determinations of $(LH)^+Cl^-$ and HgL_2Cl_2 and relevant structural information are given in Tables 1−4, and the structures are shown in Figures 1 and 2. Both structures contain four molecules per unit cell, and the water molecules present are stabilized by a mixure of hydrogen bonds and electrostatic and stacking interactions.

The structure of the uncomplexed molecule $(LH)^+Cl^-\cdot 3H_2O$ shows no unexpected features. The molecule is in the so-called *S* conformation, as has been found previously for other C(2)-substituted thiamine derivatives.[3] The torsional angles $\Phi_T = C_{5'}-C_{3,5'}-N_3-C_2$ and $\Phi_P = N_3-C_{3,5'}-C_{5'}-C_{4'}$ defining the relative orientation of pyrimidine and thiazolium rings, are 104.1(5)° and 171.9(6)°, respectively.[17] This is generally attributed to an electrostatic interaction between S(1) and O(11) accompanied by partial charge migration.[3] The observed

Table 2. Atomic Coordinates (×10^4) for $C_{19}H_{30}N_4O_8PSCl$ and $C_{38}H_{46}HgCl_2N_8O_{12}P_2S_2$

	$C_{19}H_{30}N_4O_8PSCl$				$C_{38}H_{46}HgCl_2N_8O_{12}P_2S_2$		
	x	*y*	*z*		*x*	*y*	*z*
Cl(1)	2415(1)	−2479(1)	4270(1)	Hg(1)	0	457	2500
S(1)	1501(1)	−2542(1)	6624(1)	Cl(1)	−704(1)	−905(6)	2316(2)
P(1)	1781(1)	173(1)	5641(1)	S(1)	2077(1)	191(4)	4435(2)
O(5δ)	361(1)	152(1)	5242(1)	P(1)	2293(1)	−1408(4)	2891(2)
O(5ε)	2438(1)	238(1)	4721(1)	O(11)	2015(3)	−1964(13)	5398(4)
O(5φ)	2336(1)	784(1)	6414(1)	O(5γ)	1866(2)	−380(11)	2889(4)
O(5γ)	2173(1)	−653(1)	6136(1)	O(5δ)	2724(2)	−908(10)	3538(4)
C(5β)	3476(1)	−776(1)	6725(1)	O(5ε)	2305(2)	−756(11)	2207(4)
C(5α)	3683(1)	−1635(1)	6779(1)	O(5φ)	2170(3)	−3329(10)	2822(4)
C(5)	2826(1)	−2023(1)	7316(1)	O(w)	3485(3)	160(14)	3414(4)
C(4)	2912(1)	−2035(1)	8341(1)	N(1′)	242(3)	2542(13)	3420(4)
N(3)	1895(1)	−2439(1)	8560(1)	N(3′)	97(3)	4026(13)	4266(4)
C(2)	1094(1)	−2754(1)	7732(1)	N(4α′)	660(3)	4435(14)	5407(4)
C(4α)	3961(1)	−1721(1)	9178(1)	N(3)	1656(3)	2498(13)	4746(4)
C(3,5′)	1692(1)	−2450(1)	9607(1)	C(2′)	−34(4)	3381(16)	3635(5)
C(5′)	2515(1)	−3017(1)	10335(1)	C(4′)	545(3)	3915(15)	4757(5)
C(6′)	3282(1)	−3535(1)	10044(1)	C(5′)	872(3)	3252(16)	4551(5)
N(1′)	4033(1)	−4010(1)	10747(1)	C(6′)	693(3)	2540(16)	3896(5)
C(2′)	4055(1)	−3948(1)	11767(1)	C(2α′)	−526(4)	3657(20)	3097(6)
N(3′)	3336(1)	−3467(1)	12117(1)	C(3,5′)	1382(3)	3385(17)	5052(6)
C(4′)	2530(1)	−3015(1)	11412(1)	C(2)	1816(3)	879(17)	4910(5)
N(4α′)	1752(1)	−2578(1)	11779(1)	C(5)	1962(3)	2197(15)	3999(5)
C(2α′)	4945(1)	−4445(1)	12529(1)	C(4)	1734(3)	3309(16)	4218(6)
C(10)	−14(1)	−3284(1)	7731(1)	C(4α)	1588(4)	5184(17)	3994(7)
O(11)	−598(1)	−3408(1)	6663(1)	C(10)	1786(4)	−381(18)	5432(5)
C(1″)	488(1)	−4016(1)	8315(1)	C(1″)	1286(4)	−797(15)	5233(6)
C(2″)	1298(1)	−4483(1)	7942(1)	C(2″)	1138(4)	−463(20)	5731(7)
C(3″)	1776(1)	−5159(1)	8495(1)	C(3″)	675(5)	−807(22)	5538(9)
C(4″)	1441(1)	−5348(1)	9390(1)	C(4″)	384(5)	−1437(21)	4876(10)
C(5″)	636(1)	−4873(1)	9764(1)	C(5″)	526(5)	−1765(19)	4381(8)
C(6″)	153(1)	−4204(1)	9226(1)	C(6″)	986(4)	−1389(17)	4567(7)
Ow(3)	4567(1)	−3400(1)	5781(1)	C(5α)	2095(4)	2553(17)	3435(6)
Ow(1)	1847(1)	−865(1)	3350(1)	C(5β)	1811(4)	1486(18)	2773(6)
Ow(2)	−856(1)	−1348(1)	2840(1)				

Table 3. Bond Distances (Angstroms) in $(LH)^+Cl^-\cdot 3H_2O$ and $HgL_2Cl_2\cdot H_2O$

bond	$(LH)^+Cl^-\cdot 3H_2O$	$HgL_2Cl_2\cdot H_2O$
Hg(1)−Cl(1)		2.391
Hg(1)−Cl(1A)		2.391
Hg(1)−N(1′)		2.341
Hg(1)−N(1′A)		2.341
P(1)−O(5γ)	1.606(5)	1.600(9)
P(1)−O(5ε)	1.585(8)	1.566(9)
P(1)−O(5δ)	1.505(6)	1.492(6)
P(1)−O(5φ)	1.503(5)	1.480(8)
O(5γ)−C(5β)	1.452(8)	1.414(15)
C(5β)−C(5α)	1.526(9)	1.509(16)
C(5)−C(5α)	1.491(10)	1.494(21)
S(1)−C(5)	1.756(7)	1.713(11)
S(1)−C(2)	1.698(9)	1.694(13)
C(2)−N(3)	1.338(8)	1.301(15)
N(3)−C(4)	1.415(9)	1.414(17)
C(4)−C(4α)	1.482(8)	1.486(17)
C(2)−C(10)	1.529(9)	1.503(18)
C(10)−O(11)	1.419(8)	1.424(17)
C(10)−C(1″)	1.529(9)	1.525(17)
C(1″)−C(2″)	1.393(11)	1.391(23)
C(2″)−C(3″)	1.424(10)	1.404(23)
C(3″)−C(4″)	1.382(12)	1.369(23)
C(4″)−C(5″)	1.399(11)	1.369(31)
C(5″)−C(6″)	1.406(9)	1.402(21)
C(6″)−C(1″)	1.402(11)	1.372(15)
N(3)−C(3,5′)	1.475(10)	1.500(18)
C(3,5′)−C(5′)	1.513(8)	1.511(13)
C(5′)−C(6′)	1.363(9)	1.352(15)
C(6′)−N(1′)	1.360(8)	1.348(12)
N(1′)−C(2′)	1.361(10)	1.353(18)
C(2′)−C(2α′)	1.491(10)	1.496(14)
C(2′)−N(3′)	1.323(9)	1.303(15)
N(3′)−C(4′)	1.362(8)	1.353(11)
C(4′)−N(4α′)	1.334(9)	1.319(15)
C(4′)−C(5′)	1.436(10)	1.432(19)

Table 4. Selected Bond Angles (Degrees) of $(LH)^+Cl^-\cdot 3H_2O$ and $HgL_2Cl_2\cdot H_2O$

angle	$(LH)^+Cl^-\cdot 3H_2O$	$HgL_2Cl_2\cdot H_2O$
Cl(1)−Hg(1)−N(1′)		109.9(3)
Cl(1)−Hg(1)−Cl(1A)		129.6(2)
N(1′)−Hg(1)−Cl(1A)		103.2(2)
N(1′)−Hg(1)−N(1′A)		96.5(5)
C(2)−S(1)−C(5)	91.4(4)	91.1(6)
O(5γ)−P(1)−O(5δ)	105.2(3)	109.4(5)
O(5δ)−P(1)−O(5ε)	111.5(4)	111.1(5)
O(5δ)−P(1)−O(5φ)	116.9(3)	115.6(4)
O(5γ)−P(1)−O(5ε)	105.0(3)	105.0(4)
O(5γ)−P(1)−O(5φ)	110.3(3)	105.5(5)
O(5ε)−P(1)−O(5φ)	107.3(3)	109.6(5)
P(1)−O(5γ)−C(5β)	118.3(4)	121.0(9)
Hg(1)−N(1′)−C(2′)		125.5(6)
Hg(1)−N(1′)−C(6′)		116.9(8)
C(2′)−N(1′)−C(6′)	119.4(6)	114.9(9)
C(2′)−N(3′)−C(4′)	117.7(6)	119.4(11)
C(3,5′)−N(3)−C(2)	123.3(6)	123.0(11)
C(3,5′)−N(3)−C(4)	121.9(5)	122.6(9)
C(2)−N(3)−C(4)	114.6(6)	114.3(11)
N(1′)−C(2′)−N(3′)	123.4(6)	125.4(9)
N(3′)−C(4′)−C(5′)	121.7(6)	118.9(10)
C(4′)−C(5′)−C(6′)	116.4(5)	115.8(9)
C(4′)−C(5′)−C(3,5′)	119.4(6)	120.2(10)
C(6′)−C(5′)−C(3,5′)	124.3(6)	123.9(12)
N(1′)−C(6′)−C(5′)	121.2(6)	124.8(12)
N(3)−C(3,5′)−C(5′)	113.9(6)	110.9(9)
S(1)−C(2)−N(3)	111.4(5)	112.1(9)
S(1)−C(2)−C(10)	121.7(6)	119.4(9)
S(1)−C(5)−C(5α)	121.4(5)	121.7(9)
S(1)−C(5)−C(4)	110.7(5)	111.5(10)
C(4)−C(5)−C(5α)	127.5(6)	126.8(11)
C(5)−C(5α)−C(5β)	111.8(5)	112.3(11)
O(5γ)−C(5β)−C(5α)	106.6(5)	112.7(9)
O(11)−C(10)−C(2)	103.8(6)	105.0(11)
O(11)−C(10)−C(1″)	113.0(5)	110.2(10)

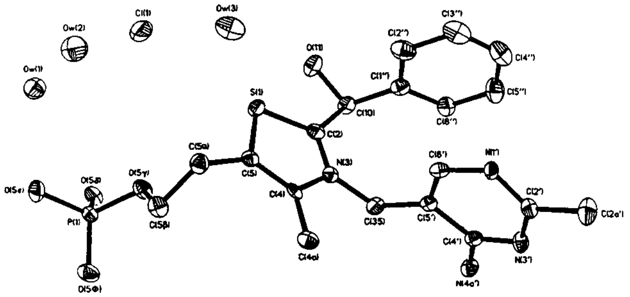


Figure 1. Crystal structure of 2-(α-hydroxybenzyl)thiamine monophosphate chloride [$(LH)^+Cl^-\cdot 3H_2O$].

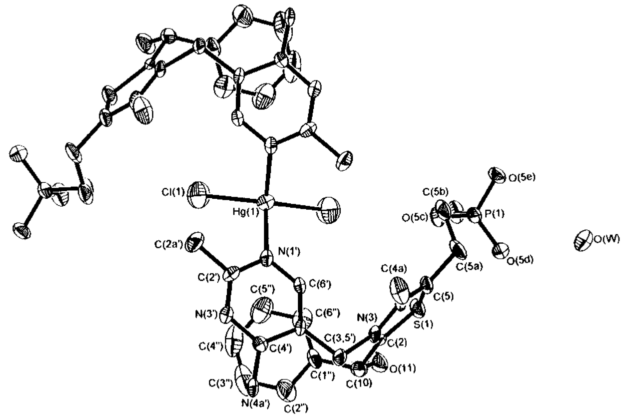


Figure 2. Crystal structure of HgL_2CL_2.

S(1)···O(11) distance in $(LH)^+Cl^-$ is 2.703(11) Å. There is a stacking interaction between the aromatic rings of the pyrimidine and the benzyl rings [average distance 3.113(9) Å]. The P(1)−O(5γ) and P(1)−O(5ε) bonds lengths are 1.606(5) and 1.585(8) Å, respectively, indicating that there is probably an O(5ε)−H bond. By contrast, the P(1)−O(5φ) and P(1)−O(5δ) bonds are significantly shorter at 1.503(5) and 1.505(6) Å, respectively, implying that these are P−O (terminal) bonds and, thus, that the phosphate group is in the monoanionic form.

A close examination of the structure of $HgL_2Cl_2\cdot H_2O$ reveals a number of interesting features. In this complex, the coordination around the mercury atom is pseudotetrahedral with Hg−Cl and Hg−N bond lengths of 2.391 and 2.341 Å, respectively, and some deviations from the purely tetrahedral bond angle. A crystallographic 2-fold rotation axis passes through the Hg atom. It is noticeable that the Hg−Cl bond length is shorter and the Hg−N bond length is longer than those in the related complex Hg[2-(α-hydroxybenzyl)thiamine]Cl_3.[11] The C(2′)−N(1′)−C(6′) bond angle of 114.9(9)° and the C(4′)−N(4α′) bond length of 1.319(15) Å in HgL_2Cl_2 are smaller than those found in other structures of protonated and metalated thiamine derivatives[3] [e.g., compare 119.4(6)° and 1.334(9) Å for $(LH)^+Cl^-$]. These, both angle and length, have previously been interpreted as being functions of the Lewis acid strength of the bonded metal for complexes of the type $MLCl_3$,[18] e.g., following the order $Zn^{2+} < Cu^+ < Cd^{2+} < Hg^{2+} < Pt^{2+}$ for the angle, but the smaller angle and length here are probably simply a reflection of the different stoichiometry.

As in the case of $(LH)^+Cl^-\cdot 3H_2O$ and other active aldehyde derivatives, the molecule is in the *S* conformation [Φ_T =

---

(13) Herzfeld, J.; Berger, A. E. *J. Chem. Phys.* **1980,** *73,* 6021.
(14) Hawkes, G. E.; Sales, K. D.; Lian, L. Y.; Gobetto, R. *Proc. R. Soc. London A* **1989,** *424,* 93.
(15) Mason, J. *Solid State Nucl. Magn. Reson.* **1993,** *2,* 285.
(16) Haeberlen, U. High Resolution NMR in Solids: Selective Averaging. *Adv. Magn. Reson. Suppl.* **1976,** *1,* 9.
(17) Pletcher, J.; Sax, M. *J. Am. Chem. Soc.* **1972,** *94,* 3998.

Table 5. Principal Components of ^{31}P, the Chemical Shift Tensor of 2-(α-Hydroxybenzyl)thiamine Monophosphate Chloride and Its Complexes with Zn(II), Cd(II), and Hg(II)[a,b]

complex	pH	δ_{iso}	δ_{11}	δ_{22}	δ_{33}	$\Delta\sigma$	η	Ω	K
L	6.0	1.2	75.2	13.0	−84.6	128.7	0.73	160.0	0.22
$(LH)^+Cl^-$	3.5	0.7	72.4	11.2	−81.5	123.3	0.74	153.8	0.21
$ZnLCl_2$	6.0	0.5(br)	57.6	−5.7	−49.8	−85.3	0.78	107.4	−0.18
$Zn(LH)Cl_3$	3.5	0.3(w)	59.9	7.3	−67.9	101.5	0.78	127.8	0.16
		−2.3	68.6	10.4	−85.9	125.3	0.70	154.4	0.25
		−4.1	65.6	14.1	−92.0	131.9	0.5	157.7	0.35
$CdLCl_2$	6.0	2.9(br)	60.1	−0.9	−51.1	−86.1	0.87	111.2	−0.10
		1.7	68.3	11.7	−74.9	114.9	0.74	143.2	0.21
		0.0(w)[c]							
		−1.4(w)[c]							
HgL_2Cl_2 (powder)	6.0	2.4	61.0	14.9	−68.7	106.7	0.65	129.7	0.29
HgL_2Cl_2 (crystals)	6.0	2.4	59.6	15.9	−67.9	105.6	0.62	127.5	0.32

[a] The chemical shifts are given in ppm relative to 85% H_3PO_4 using the convention $\delta_{11} > \delta_{22} > \delta_{33}$. The other parameters quoted are defined in the Experimental Section. [b] The isotropic chemical shifts are accurate within 0.2 ppm, except for the peaks marked as broad (br) for which the accuracy is within 1 ppm. The estimated uncertainties in the shielding tensor components are within ±1.5 ppm for the strong narrow peaks, ±3 ppm for $ZnLCl_2$, and ± 6 ppm for the broad overlapped resonance in $CdLCl_2$. [c] Weak peaks (w) were not simulated due to the extent of peak overlap.

−98.1(9)°, Φ_P = 176.1(10)°]. As a consequence, the S(1)···O(11) distance is 2.711(14) Å, which is the shortest found thus far for substituted thiamine complexes with divalent cations. This is important as, according to Hogg,[19] the S(1)···O(11) electrostatic interaction is responsible for the O−H proton liberation during the enzymatic process. As for $(LH)^+Cl^-\cdot 3H_2O$, there is also a stacking interaction between the pyrimidine and benzyl aromatic rings [average distance 3.371(13) Å]. The angle between the pyrimidine and thiazolium planes in $HgL_2Cl_2\cdot H_2O$ is 73.3(8)°, which again is smaller than that found for related complexes [e.g., compare 80.4(6)° for $(LH)^+Cl^-\cdot 3H_2O$]. This may be of importance as it has been suggested that those complexes with smaller angles between these planes are more susceptible to oxidation from thiamine to thiochrome.[3]

$HgL_2Cl_2\cdot H_2O$ contains two long P−O distances and two short P−O distances with the latter attributed to P−O (terminal) bonds, implying that, as in the case of $(LH)^+Cl^-$, the phosphate group is in the monoanionic form (see Table 3).

Solid-State ^{31}P NMR Spectroscopy. There have been a number of studies correlating ^{31}P chemical shift tensors with structural parameters, and it is normally possible to distinguish between monoanionic and dianionic phosphate environments fairly easily on the basis of their anisotropy.[20,21] Figure 3 shows spectra of the uncomplexed phosphate ester and its zinc and cadmium complexes. Both the full spinning sideband manifold at slow spinning speeds and the isotropic peak region at fast spinning speeds are shown. Analysis of the slow spinning speed spectra gives the principal components of the ^{31}P shielding tensor reported in Table 5.

The uncomplexed molecules L and $(LH)^+Cl^-$ prepared at pH 6.0 and 3.5, respectively, give identical anisotropy parameters within experimental error. The anisotropies observed (large positive $\Delta\sigma$ values) are characteristic of monoanionic phosphate enviroments.[20−22] For instance, the monoanionic form of the 2′-phosphate group in NADPH has been found to have anisotropy parameters $\Delta\sigma$ = 130 ppm and η = 0.56.[22] This assignment of the rate of the phosphate group is in agreement with the interpretation of the P−O bond lengths derived from the crystal structure discussed earlier.

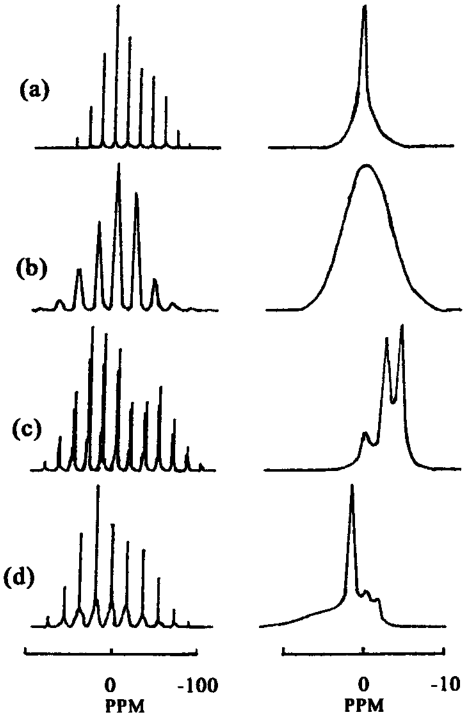


Figure 3. ^{31}P CP/MAS NMR spectra of (a) L, pH 6; (b) $ZnLCl_2$, pH 6; (c) $Zn(LH)Cl_3$, pH 3.5; and (d) $CdLCl_2$, pH 6. The spinning speeds were 2−2.6 kHz for the slow spinning spectra (left-hand side) and 11.2 kHz for the fast spinning spectra (right-hand side; an expansion of only the center band is shown).

A single peak, though rather broad, is observed for $ZnLCl_2$. While the isotropic chemical shift is similar to that observed for the uncomplexed molecule, the anisotropy parameters are very different. This implies a difference in electronic shielding at the ^{31}P nucleus and so indicates a major change in the local phosphorus environment. The negative values of $\Delta\sigma$ and κ suggest that dianionic phosphate is present;[20,22] however, the asymmetry parameter, η, is rather higher than expected for a dianionic phoshate environment. Thus, we believe that the ^{31}P shielding tensor components provide clear evidence for direct binding of the metal to the phosphate group. Literature values of ^{31}P shielding tensors of phosphates covalently bonded to metals are not available to our knowledge.

The zinc complex prepared at pH 3.5 [$Zn(LH)Cl_3$] gives three peaks in its^{31}P NMR spectrum. Two major peaks are observed, and these may well be due to crystallographically different phosphorus sites in the same phase, while the third peak is weak and so is probably just a minor impurity. Both of the major peaks have shielding anisotropies comparable to that of the

---

(18) Cramer, R.; Maynard, R.; Evangelista, R. *J. Am. Chem. Soc.* **1984,** *106,* 111.

(19) Hogg, J. *Bioorg. Chem.* **1988,** *10,* 233.

(20) Olivieri, A. *J. Magn. Reson.* **1990,** *88,* 1.

(21) Un, S.; Klein, M. P. *J. Am. Chem. Soc.* **1989,** *111,* 5119.

(22) Gerothanassis, I. P.; Barrie, P. J.; Birdsall, B.; Feeney, J. *Eur. J. Biochem.* **1994,** *226,* 211.

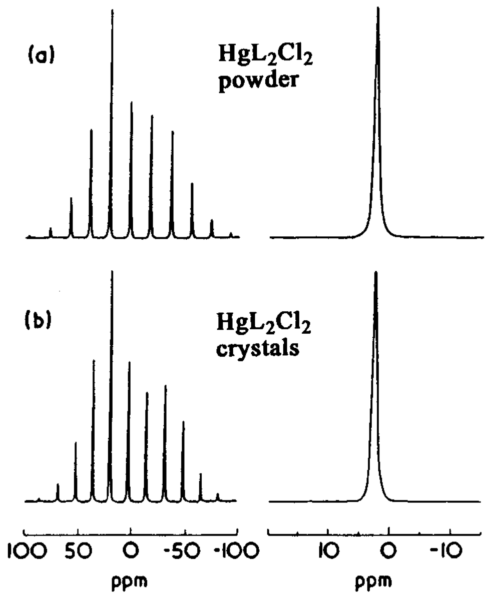


Figure 4. ^{31}P CP/MAS NMR spectra of (a) HgL_2CL_2, powdered sample (spinning speed = 2290 Hz) and (b) HgL_2CL_2 crystals as used in the single-crystal X-ray structure determination (spinning speed = 2030 Hz).

uncomplexed phosphate ester, thus clearly indicating that no metal binding to the phosphate group occurs at this pH.

The cadmium complex, $CdLCl_2$, prepared at pH 6 gives a broad peak (ca. 58% of total area), a strong narrow peak (ca. 36%), and two weak narrow peaks. Analysis of the sidebands from the broad peak gives tensor components similar to those of $ZnLCl_2$, in which direct metal−phosphate bonding was deduced. On the other hand, the chemical shift tensor of the strong narrow peak is similar to that of the uncomplexed phosphate. Hence, this sample appears to be a mixure of two phases: one with cadmium directly bonded to the phosphate group and one with cadmium bonded elsewhere [probably the N(1′) group of the pyrimidine ring].

Two samples of HgL_2Cl_2 were analyzed by ^{31}P CP/MAS NMR spectroscopy (see Figure 4). The first of these was a polycrystalline powder prepared by precipitation from aqueous solution, while the second was a batch of the crystals used in the single-crystal X-ray structure determination (reported earlier in this paper). In contrast to the situation for $ZnLCl_2$ and $CdLCl_2$, both samples of HgL_2Cl_2 give a single sharp ^{31}P resonance. Analysis of the spinning sideband intensities revealed that the two samples have identical tensor components, as reported in Table 5. The shielding anisotropy parameters are indicative of monoanionic phosphate and show little difference from the uncomplexed molecule, indicating that mercury does not bind directly to the phosphate group. This is in agreement with the single-crystal X-ray structure determination discussed earlier, which showed that the mercury binds only to the N(1′) group of the pyrimidine ring. Further, the NMR results are indicative of phosphate in the monoanionic form, which is in agreement with the fact that the crystal structure shows P−O distances characteristic of two P−O (terminal) bonds.

It is worth mentioning that the ^{31}P NMR spectra and analyses were in fact performed before suitable crystals of HgL_2Cl_2 became available for structure determination. The prediction based on NMR results that mercury did not bind to the phosphate group was subsequently confirmed by the crystal structure. The prediction that metal phosphate bonding does occur at pH 6 for the zinc complex and for one component of the cadmium mixture prepared at pH 6 has not been confirmed by crystallography, as attempts to prepare suitable crystals have not been successful. It is, however, an indication of the power of ^{31}P solid-state NMR spectroscopy that such a prediction can be made for these complexes in the absence of other data.

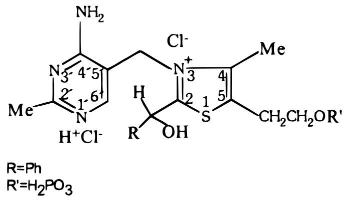
Table 6. Selection of Isotropic ^{13}C Chemical Shifts of 2-(α-Hydroxybenzyl)thiamine Chloride, $(LH)^+Cl^-$, and Its Complexes with Zn^{2+}, Cd^{2}, and Hg^{2+} (δ in ppm from TMS)

compound	pH	C(2′)	C(6′)	C(5β)
$(LH)^+Cl^-$	3.5	162.1	129.7	63.6
L	6	163.2	128.6	63.1
$ZnLCl_2$	6	167.3	130.5	65.1
$CdLCl_2$	6	166.3	130.3	64.9
$Zn(LH)Cl_3$	3.5	164.4	130.5	67.2
$Cd(LH)Cl_3$	3.5	164.1	130.3	67.1
$(ZnCl_4)^{2-}(LH)_2^+$	1	161.9	129.7	67.5
$(CdCl_4)^{2-}(LH)_2^+$	1	162.6	129.6	67.3
$(HgCl_4)^{2-}(LH)_2^+$	1	162.6	129.8	67.3
HgL_2Cl_2	6	167.1	131.6	65.9

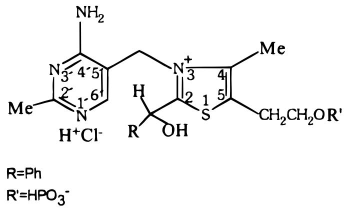
Solid-State ^{13}C NMR Spectroscopy. ^{13}C solid-state NMR spectroscopy has developed into a routine complement to X-ray crystallography, and it can, in favorable cases, distinguish between different protonation and/or metalation states.

Chemical shifts of selected carbon atoms are included in Table 6. Assignments were based on comparisons with previous results reported on complexes with 2-(α-hydroxybenzyl)-thiamine[12] and cross-polarization interrupted-decoupling experiments.[23]

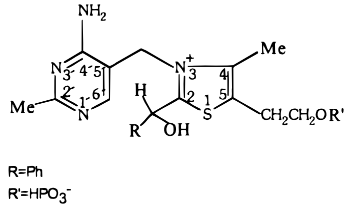
Comparison of the spectra of the ligand at pH's 3.5 [$(LH)^+Cl^-$] and 6 (L) reveals that it is protonated at N_1' and deprotonated at the phosphate group at pH 3.5 and deprotonated at both sites at pH 6. Thus, the carbon atoms near N_1' of $(LH)^+Cl^-$, i.e., C_2' and C_6', appear at 162.1 and 129.7 ppm, respectively. For L, however, they shift downfield and upfield by 1.1 ppm, respectively. The $C_{5\beta}$ atom near the phosphate group, on the other hand, occurs at 63.6 ppm for $(LH)^+Cl^-$ and 63.1 ppm for L, respectively. The ligand forms are presented here:



pH=1



pH=3.5



pH=6

Addition of MCl_2 (M = Zn^{2+}, Cd^{2+}, Hg^{2+}) to a freshly prepared solution of the ligand at pH 1 results in the precipitation of the corresponding $(MCl_4)^{2-}(LH)_2^+$ complexes.

Table 7. Characteristic IR and Raman Bands (cm^{-1}) of 2-(α-Hydroxybenzyl)thiamine Monophosphate Chloride, $(LH)^+Cl^-$, and Its Complexes with Zn^{2+}, Cd^{2+}, and Hg^{2+}

		Raman				IR			
compound	pH	δNH_2	$\nu C{=}N$	$\nu P{-}O$	$\nu M{-}Cl$	δNH_2[a]	$\nu C{=}N$	$\nu P{-}O$	$\nu M{-}Cl$
$(LH)^+Cl^-$	3.5	1654, 1620	1599	1174		1675 (1185)	1658	1160	
L	6					1670 (1185)	1610	1130	
$ZnLCl_2$	6	1620	1602	1160	291	1655	1625	1150	290m
$Zn(LH)Cl_3$	3.5	1660, 1624	1601	1188	323, 300, 290	1690, 1660 (1170)	1620	1200	320, 285
$(ZnCl_4)^{2-}(LH)_2{}^+$	1	1657, 1620	1601	1188	338, 304	1680 (1170)	1658	1215	295
$CdLCl_2$	6	1630	1602	1159	280, 262	1660	1622	1155	282
$Cd(LH)Cl_3$	3.5	1625	1600	1181	283	1688, 1660sh	1620	1175	328, 285
$(CdCl_4)^{2-}(LH)_2{}^+$	1	1660	1602	1187	259	1675 (1185)	1660	1215	255
$(HgCl_4)^{2-}(LH)_2{}^+$	1	1661	1601	1187	262	1680 (1205)	1660	1217	270, 255
HgL_2Cl_2	6	1618	1603	1159	277	1697 (1205)	1620	1158	270

[a] The numbers in parentheses are the δND_2 vibrations.

For the complexes $ZnLCl_2$ and $CdLCl_2$, prepared at pH 6, the C_2' shifts downfield by 3−4 ppm and the C_6' shifts by about 2 ppm, compared to the deprotonated ligand (L) at the same pH. Also, the $C_{5\beta}$ atom adjacent to the phosphate group shifts downfield by about 2 ppm, again compared to L. These results strongly indicate simultaneous bonding of the metals at N_1' and the phosphate group.

In the series $Zn(LH)Cl_3$ and $Cd(LH)Cl_3$, prepared at pH 3.5, again C_2' and C_6' shift upfield by ∼1 ppm and downfield by ∼2 ppm, respectively, compared to L (Table 6), again implying metalation at N_1'. The chemical shift of the $C_{5\beta}$ atom near the phosphate group, on the other hand, appears near 67.00 ppm. These complexes therefore may have a zwitterionic structure with the $MCl_3{}^-$ moiety bonded to N_1' and the phosphate group protonated.[3,11,24]

The series $(MCl_4)^{2-}(LH)_2{}^+$, on the other hand, prepared at pH 1, shows that C_2', C_6', and $C_{5\beta}$ chemical shifts comparable to the protonated ligands may rather be ionic salts.[4,25,26]

Finally, in the complex HgL_2Cl_2 containing $Hg{-}N_1'$ bonds and a deprotonated monoanionic phosphate group, as was also shown by the X-ray crystal structure, the C_2' appears at 167.1 ppm, shifted downfield by 3.9 ppm, and the C_6' appears at 131.6 ppm, shifted downfield by 3.00 ppm, compared to L.

Vibrational Spectroscopy. Bands that can be assigned to δNH_2 and $\nu C{=}N$ of the pyrimidine ring and $\nu P{-}O$ and $\nu M{-}Cl$ of the ligand and the complexes, in the IR and Raman spectra, are included in Table 7. In the region 1600−1700 cm^{-1} of the IR spectra, both δNH_2 and $\nu C{=}N$ of pyrimidine are expected. In fact, deuteration experiments have shown that the $\nu C{=}N$ of the pyrimidine ring of $(LH)^+Cl^-$ occurs at 1658 cm^{-1}, while the δNH_2 occurs at 1675 cm^{-1}. The same frequencies appear at 1610 and 1670 cm^{-1}, respectively, for L (Table 7). The $\nu C{=}N$ band is shown near 1660 cm^{-1} whenever the N_1' is protonated, i.e., $(LH)^+Cl^-$ and $(MCl_4)^{2-}(LH)^+{}_2$ ($M = Zn^{2+}$, Cd^{2+}, Hg^{2+}), and near 1620 cm^{-1} when it is metalated, also following the order $Hg^{2+} < Cd^{2+} < Zn^{2+} < H^+$, as in the case of the same complexes with 2-(α-hydroxybenzyl)thiamine.[11,27]

Evidence for the metalation or protonation of the phosphate group, on the other hand, is given from the positions of the $\nu P{-}O$ bands, occurring at lower frequencies (1130−1160 cm^{-1}) when it is deprotonated and metalated and at higher frequencies (1175−1215 cm^{-1}) when it is protonated.[28] Similar behavior is also observed for the band at the same range of frequencies of the Raman spectra, again assigned to $\nu P{-}O$ (see Table 7).

Finally, assignments for the $\nu M{-}Cl$ bands are also included in Table 7.

Conclusions

The binding sites of Zn^{2+}, Cd^{2+}, and Hg^{2+} in complexes with 2-(α-hydroxybenzyl)thiamine monophosphate chloride, $(LH)^+Cl^-$, have been explored. Both the N(1′) site and the phosphate group may be metal-binding sites of importance in determining the enzymatic action of the ligand. The crystal structure of HgL_2Cl_2 indicates that the *S* conformation is adopted, with metal binding solely to the N(1′) group of the pyrimidine ring. Metal binding to the phosphate group is inferred from the ^{31}P NMR spectra of $ZnLCl_2$ and one component of the $CdLCl_2$ sample. ^{31}P NMR spectra and the other techniques allow one to conclude the protonation states of the phosphate group. In general, it seems that ^{31}P shielding tensors will be useful in characterizing the binding of metal cations to the phosphate group in molecules of biological interest.

Supporting Information Available: Tables of elemental analyses of the complexes, anisotropic displacement coefficients, hydrogen atom coordinates, electrostatic contacts and hydrogen-bonding interactions, best weighted least-squares planes for pyrimidine, thiazole, and benzyl rings, and various torsional angles for both $(LH)^+Cl^-\cdot 3H_2O$ and $HgL_2Cl_2\cdot H_2O$ (16 pages). Ordering information is given on any current masthead page.

IC950758X

---

(23) Xialing, W.; Shanmin, Z.; Xaewen, W. *J. Magn. Reson.* **1988,** *77,* 343.

(24) Hadjiliadis, N.; Markopoulos, J.; Pneumatikakis, G.; Katakis, D.; Theophanides, T. *Inorg. Chim. Acta* **1977,** *25,* 21.

(25) Hadjiliadis, N.; Yannopoulos, A.; Bau, R. *Inorg. Chim. Acta* **1983,** *69,* 109.

(26) Cramer, R. E.; Kirkup, R. E.; Carrie, M. J. *Inorg. Chem.* **1988,** *27,* 123.

(27) Hadjiliadis, N.; Louloudi, M.; Butler, I. S. *Spectrochim. Acta* **1991,** *47A,* 445.

(28) Khalil, V. J.; Brown, T. I. *J. Am. Chem. Soc.* **1964,** *86,* 5113.