Regio-Directed Synthesis of a $Zn^{II}Fe^{III}$ Complex from an Unsymmetrical Ligand and Its Relevance to Purple Acid Phosphatases

Catherine Belle,*[a] Isabelle Gautier-Luneau,[a] Lydia Karmazin,[a] Jean-Louis Pierre,[a] Sabine Albedyhl,[b] Bernt Krebs,[b] and Michel Bonin[c]

Keywords: Heterometallic complexes / Zinc / Iron / Unsymmetrical ligands / Phosphatases

A new heterodinuclear $Zn^{II}Fe^{III}$ μ-phenoxy complex has been prepared and isolated from the unsymmetrical ligand 2-{[bis(2-hydroxybenzyl)]amino}methyl-6-{[2-pyridinylmethyl)amino]methyl}-4-methylphenol (H_3L^1), which provides two distinct N- and O-rich donor sets. The regioselective complexation is confirmed in solution by ^1H NMR studies (comparison of the spectra obtained from H_3L^1 and HL^2 – a ligand with only the N-donor set available – and a zinc salt). The crystal structure of the bis(μ-diphenylphosphato)$Zn^{II}Fe^{III}$ complex $[ZnFeL^1\{O_2P(OPh)_2\}_2]$ (**1**) is reported. The NO_5 coordination sphere around the Fe^{III} reproduces, for the first time, the oxygen-rich coordination sphere around iron(III) in red kidney bean purple acid phosphatase (KBPAP).

Introduction

Purple acid phosphatases (PAPs) are non redox-active dinuclear metalloenzymes representing a group of widely distributed phosphomonoesterases. Purple acid phosphatases require acidic conditions for optimum activity and are characterized by an intense purple color ($\lambda_{max} \approx 550$ nm) due to a charge-transfer transition from a tyrosine group to the iron(III).[1] Mammalian PAPs contain an $Fe^{II}Fe^{III}$ center at the active site while the most studied plant enzyme, isolated from red kidney bean (KBPAP), contains a $Zn^{II}Fe^{III}$ center. The X-ray structure of KBPAP (resolution 2.65 Å) shows that the two metal ions are 3.26 Å apart and bridged by Asp_{164}; each metal center has octahedral coordination.[2] The iron is further coordinated by Tyr_{167}, His_{325} and Asp_{135} residues, and zinc by His_{323}, His_{286} and Asn_{201} residues (Figure 1A). Three exogenous ligands are also included in the X-ray model to complete the coordination sphere: one terminal hydroxy ligand to iron, one terminal water ligand to zinc, and one hydroxy bridge between the metal ions. An important role is assigned to such unsymmetrical coordination, providing selective substrate binding and stereochemical control during the catalytic cycle.[2] The crystal structure of KBPAP with a coordinated μ-phosphate (the product of the hydrolysis reaction) (resolution 2.7 Å; Figure 1B) or with a μ-tungstate inhibitor (resolution 3.0 Å) have also been reported.[2b] Phosphate or tungstate oxoanions bind in a bidentate bridging mode to the two metals ions. The intermetallic distances have been refined to 3.33 Å and 3.20 Å, respectively.

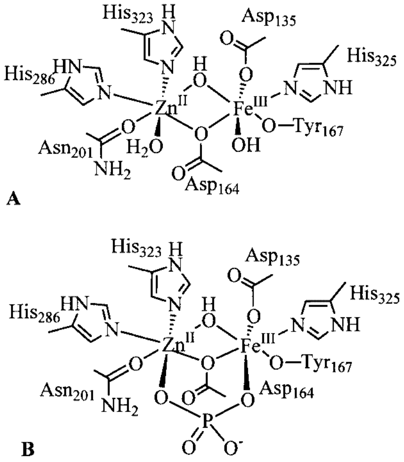


Figure 1. The active site of kidney bean purple acid phosphatase (KBPAP) (A) and of its phosphate adduct (B) from X-ray crystallography[2b]

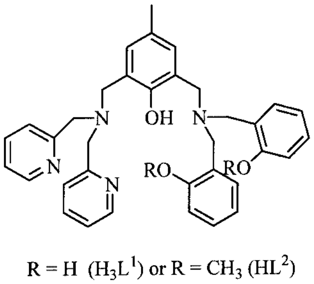
Although the metal ions in KBPAP are in two chemically distinct environments, the heterodinuclear $Zn^{II}Fe^{III}$ complexes described as models for the active site of KBPAP[3−8] have been prepared from symmetrical ligands. Only limited examples of unsymmetrical $Zn^{II}Fe^{III}$ complexes have been

---

[a] LEDSS, Chimie Biomimétique, UMR CNRS 5616, Université J. Fourier,
B. P. 53, 38041 Grenoble Cedex, France
Fax: (internat.) +33-47/651-4836
E-mail: Catherine.Belle@ujf-grenoble.fr
[b] Anorganisch-Chemisches Institut, Westfälische Wilhelms-Universität
Wilhelm-Klemm-Straβe 8, 48149 Münster Germany
[c] Institute of Crystallography, University of Lausanne, BSP, 1015 Lausanne, Switzerland

reported. They involve constitutionally unsymmetrical ligands with an alkoxy bridge but with identical chemical environments (two N_3O coordination spheres) around the metal ions.[9,10] The only example characterized by an X-ray structure has iron and zinc atoms bridged by an arsenate group[10] and with two different coordination environments (N_3O_3 and N_3O_2 for Fe and Zn, respectively). Currently, there is great interest in the development of new $Zn^{II}Fe^{III}$ complexes which may be used as models for enzyme-substrate/inhibitor intermediates.

We have used the known binucleating ligand H_3L^1 (Scheme 1),[11] which involves two different coordination sites connected by a phenol spacer: a hard site {a [bis(2-hydroxybenzyl)amino]methyl moiety} suited for iron(III) complexation and a softer site {a [bis(2-pyridinylmethyl)-amino]methyl moiety}, suited for zinc(II) complexation. We report here a regiodirected preparation of a heterodinuclear $Zn^{II}Fe^{III}$ complex and the X-ray structure of a diphenylphosphate adduct.



Scheme 1

Results and Discussion

Mononuclear zinc(II) complexes were prepared in solution in an NMR tube with either H_3L^1 or HL^2. HL^2 is the bis-methoxy derivative of H_3L^1 (Scheme 1)[12] where the terminal phenolate donor set has been suppressed and only one coordination site remains for metal ion complexation. The zinc complex of this ligand shows spectroscopic features for the complexation of Zn^{2+} by a bis(2-pyridinylmethyl)amino moiety. The complexation processes were monitored by ^1H NMR spectroscopy. The ligand resonances shifted progressively upon addition of a solution of $Zn(ClO_4)_2{\cdot}6H_2O$ in CD_3CN (Figure 2). Interestingly, the spectrum recorded for solutions containing a mixture of the ligand and the complex show that the two species are in slow exchange on the NMR time scale. Although different species are observed during the addition of the zinc salt only one species is present after addition of one molar equivalent of the zinc salt. The spectrum remains unchanged after the addition of a further equivalent of the zinc salt. The mononuclear zinc complexes prepared independently from H_3L^1 and HL^2 or obtained upon addition of the zinc salt to the ligand in the NMR tube are the same, as seen by their NMR spectra. Comparison of the spectra obtained with one molar equivalent of zinc salt and H_3L^1 instead of HL^2 reveals the similarity of the environment

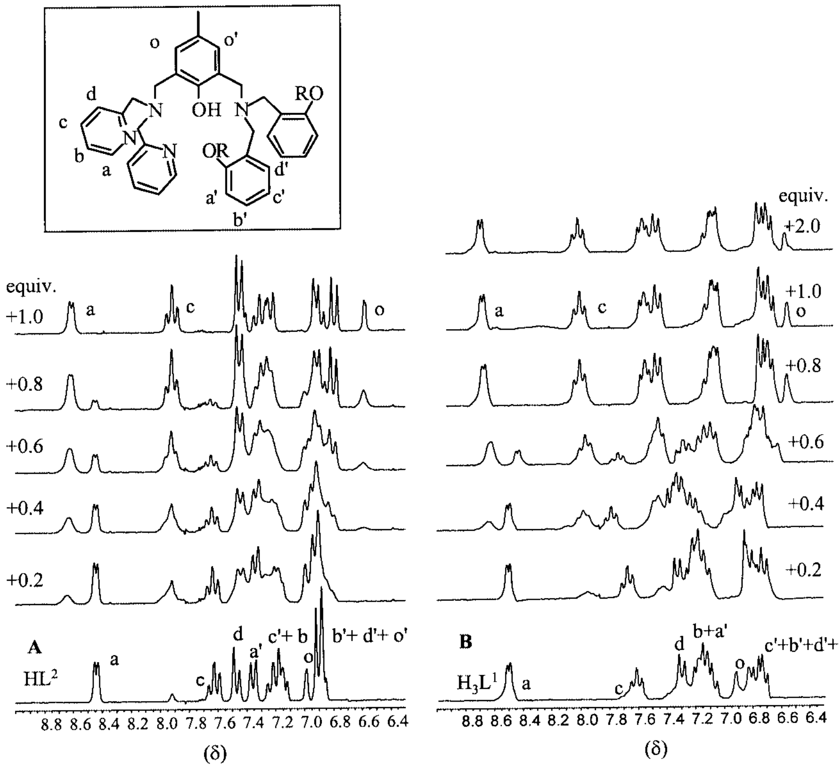


Figure 2. ^1H NMR spectra (200 MHz, CD_3CN) monitoring the formation of the mononuclear zinc complexes in the aromatic region, from HL^2(A) and H_3L^1(B) upon progressive addition of the zinc perchlorate salt; inset: ligand numbering scheme

around the zinc(II) center in the two mononuclear complexes (Figure 2). In particular, the well-resolved *ortho*- and *para*-pyridinyl resonances (protons *a* and c respectively in Figure 2) show the same behavior. In a similar manner, the resonance for the proton in the *ortho* position on the central phenyl ring and close to the pyridyl arms (signal *o* on Figure 2) appears near $\delta = 7.0$ ppm for both free ligands and is shifted to $\delta = 6.7$ ppm after complexation. Accordingly, it appears that the zinc ion is regioselectively complexed by the bis-pyridyl site of the dinucleating ligand H_3L^1.

The neutral heterobinuclear $[ZnFeL^1\{O_2P(OPh)_2\}_2]$ (**1**) complex was obtained from H_3L^1 by stepwise addition (in methanol) of zinc(II) perchlorate (1 equiv.) followed by addition of triethylamine (3 equiv.), and iron(III) perchlorate (1 equiv.). After addition of diphenylphosphate (2 equiv.) complex **1** precipitated as a violet powder. Recrystallisation by vapor diffusion of methanol into an acetonitrile solution of **1** gave crystals suitable for an X-ray diffraction study. The electronic absorption spectrum of **1** in CH_3CN displays a main feature at 501 nm ($\varepsilon \approx 3077$ M^{-1} cm^{-1}). Comparison of this spectrum with those of iron(III) complexes of μ-phenolato ligands leads to the assignment of the latter band to charge-transfer transitions from the terminal and the bridging phenolates to the iron(III).[4] The native Zn^{II}-Fe^{III} red kidney bean phosphatase displays a visible spectrum with a λ_{max} of 560 nm ($\varepsilon \approx 3360$ M^{-1} cm^{-1}).[13]

The crystal structure of **1** reveals that the two metallic atoms are triply bridged by the phenoxo group (O1) of the ligand and two μ-diphenylphosphato groups leading to the intermetallic distance of 3.7402(7) Å. An ORTEP view is shown on Figure 3, and selected bond lengths and angles are given in Table 1. The octahedral coordination geometry of the metal centers is completed by the tertiary amine (N2) and two pyridine nitrogens (N3 and N4) for Zn^{II} and by the tertiary amine (N1) and two phenolate oxygens (O2 and O3) for Fe^{III}. The distances in the $[NO_5]$ coordination sphere of the iron(III) are in the range of 1.918(2) to 2.222(3) Å and are more asymmetric than those of Zn^{II} for which the distances in the $[N_3O_3]$ coordination sphere are ranged between 2.037(3) and 2.182(3) Å. The Fe−O(phenolate) bond lengths [1.918(2) and 1.919(2) Å] are slightly longer than in the $[Fe_2L^1(\mu\text{-}OMe)(OMe)]^+$ complex[14] [1.872(5) and 1.850(5) Å] where the iron has a pentacoordinate coordination sphere. The Zn···Fe distance of 3.7402(7) Å is slightly longer than in other complexes where the metals are linked by three bridges, one phenoxy and two μ-diphenylphosphate groups. In $[ZnFe(BPMP)\{O_2P(OPh)_2\}_2]^{2+}$ and $[ZnFe(BPMOP)\{O_2P(OPh)_2\}_2]^{2+}$ the intermetallic distances are 3.695(1) Å[3] and 3.7030(7) Å,[5] respectively.

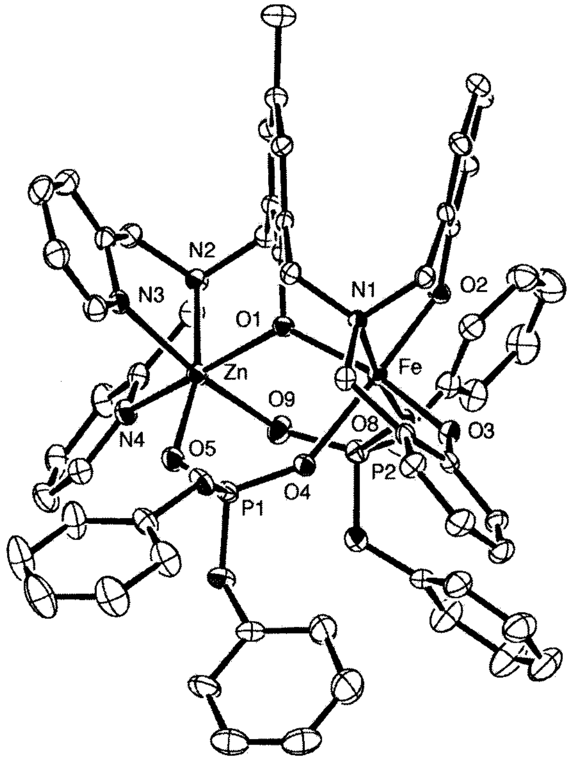


Figure 3. ORTEP plot of $[ZnFeL^1(O_2P(OPh)_2]_2]$ (**1**), with atom labels and numbering scheme; hydrogens are omitted for clarity; ellipsoids are drawn at the 30% probability level

Table 1. Selected bond lengths (Å) and angles (°) for **1**

Zn−Fe	3.7402(7)		
Zn−O1	2.124(2)	Fe−O1	2.035(3)
Zn−O5	2.037(3)	Fe−O8	2.005(2)
Zn−O9	2.130(3)	Fe−O4	2.112(2)
Zn−N2	2.182(3)	Fe−N1	2.222(3)
Zn−N3	2.180(3)	Fe−O2	1.919(2)
Zn−N4	2.151(3)	Fe−O3	1.918(2)
P1−O4	1.481(3)	P2−O8	1.484(2)
P1−O5	1.478(3)	P2−O9	1.468(3)
Zn−O1−Fe	128.1(1)		
O1−Zn−N2	91.7(1)	O1−Fe−N1	87.5(1)
O1−Zn−N4	165.5(1)	O1−Fe−O3	165.6(1)
O1−Zn−N3	89.0(1)	O1−Fe−O2	93.8(1)
O1−Zn−O9	88.2(1)	O1−Fe−O8	95.1(1)
O1−Zn−O5	96.6(1)	O1−Fe−O4	82.4(1)
O5−Zn−N2	167.3(1)	O2−Fe−N1	88.5(1)
O5−Zn−N3	91.5(1)	O2−Fe−O4	174.6(1)
O5−Zn−N4	96.0(1)	O2−Fe−O8	87.3(1)
O5−Zn−O9	97.5(1)	O3−Fe−O2	98.8(1)
O9−Zn−N4	82.9(1)	O3−Fe−N1	86.0(1)
O9−Zn−N3	170.8(1)	O3−Fe−O4	85.4(1)
O9−Zn−N2	92.3(1)	O3−Fe−O8	92.3(1)
N3−Zn−N2	79.0(1)	O4−Fe−N1	95.1(1)
N4−Zn−N3	98.0(1)	O8−Fe−N1	175.2(1)
N4−Zn−N2	77.2(1)	O8−Fe−O4	89.3(1)
Zn−O5−P1	124.0(1)	Fe−O4−P1	147.1(2)
Zn−O9−P2	139.5(2)	Fe−O8−P2	131.7(2)
O4−P1−O5	119.0(1)	O8−P2−O9	119.7(2)

Although the Zn−O1 [2.124(2) Å] and Fe−O1 [2.035(3) Å] bond lengths in complex **1** are similar to those observed in the previously cited complexes, the Zn−O1−Fe bond angle is larger [128.1(1)°] $\{[ZnFe(BPMP)\{O_2P(OPh)_2\}_2]^{2+}$: Zn−O1 = 2.176(2) Å, Fe−O1 = 2.031(2) Å, Zn−O1−Fe = 122.8(1)°; $[ZnFe(BPMOP)\{O_2P(OPh)_2\}_2]^{2+}$: Zn−O1 = 2.168(2) Å, Fe−O1 = 2.047(2) Å, Zn−O1−Fe = 122.9(1)°}. Moreover, the Zn−O1−Fe angle is similar to that observed in $[ZnFe(BPBP)(MoO_4)_2]$[8] [127.4(2)°] where, due to the longer Mo−O distance [average 1.788 Å, compared to the P−O distance (average 1.478

Å)], the Fe−O1 [2.156(5) Å] and Zn−O1 [2.104(5) Å] bonds are longer, leading to an intermetallic distance of 3.819(4) Å. All these structures indicate a remarkable structural flexibility of the intermetallic distance, which may be important for the catalytic activity. In native KBPAP with one coordinated phosphate, the Fe···Zn distance has been refined to 3.33 Å.[2b] The bridging mode of the phosphate is nearly symmetrical (with Fe−O−P and Zn−O−P angles of 120° and 127° respectively), whereas in complex **1** the angles are larger and ranged between 124.0(1)° and 147.1(1)°. Larger angles are also observed in $[ZnFe(BPMP)\{O_2P(OPh)_2\}_2]^{2+}$ and in $[ZnFe(BPMOP)\{O_2P(OPh)_2\}_2]^{2+}$ complexes.

Conclusion

The use of an unsymmetrical ligand possessing both a hard and a softer coordination site may be considered as an interesting strategy to prepare heterobinuclear complexes in a directed, non-statistical way. This paper describes a predictable and controlled synthesis of a $Zn^{II}Fe^{III}$ complex. The zinc was complexed first and solution studies revealed that zinc(II) is bound exclusively by the N-rich donor set.; the O-rich donor remains available for the subsequent complexation of iron(III). This regiocontrolled synthesis has allowed us to describe and characterize the first example of a phosphate-bridged species with a $Zn^{II}Fe^{III}$ core in an unsymmetrical, octahedral environment. The NO_5 coordination sphere around the iron(III) models the oxygen-rich coordination sphere for this ion in KBPAP.

Experimental Section

H_3L^1 and HL^2 were synthesized as reported previously.[11−12]

$[ZnFeL^1\{O_2P(OPh)_2\}_2]$ (1): A solution of $Zn(ClO_4)_2{\cdot}6H_2O$ (0.0664 g, 0.178 mmol) in methanol (3 mL) was added dropwise to a solution of H_3L^1 (0.100 g, 0.178 mmol) dissolved in methanol (4 mL). The solution was stirred for 1 h and NEt_3 (0.712 mmol) was added. After 15 min a solution of $Fe(ClO_4)_2{\cdot}9H_2O$ (0.0632 g, 0.178 mmol) in methanol (3 mL) was added, and the solution, which turned dark-red, was stirred for 1 h. Diphenylphosphate (0.0893 g, 0.356 mmol) dissolved in methanol (4 mL) was then added, and the solution turned dark and a purple powder precipitated. This precipitate was collected by filtration (79.2 mg, 38%). Crystals of X-ray quality were obtained by vapor diffusion of methanol into an acetonitrile solution of **1**. $C_{59}H_{53}FeN_4O_{11}P_2{\cdot}Zn{\cdot}CH_3OH$: calcd. C 59.59, H 4.75, Fe 4.62, N 4.63, P 5.12, Zn 5.41; found C 59.49, H 4.47, Fe 4.62, P 5.13, N 4.69, Zn 5.00. UV/Vis (CH_3CN): λ_{max} = 501 nm (ε = 3077 M^{-1} cm^{-1}). FAB-MS (NBA): *m*/*z* = 1176 $[M + H]^+$.

The mononuclear zinc(II) complexes were isolated as pale yellow powders after addition of diethyl ether to a methanol solution of $Zn(ClO_4)_2{\cdot}6H_2O$ (1 equiv.) and H_3L^1 (or HL^2) (1 equiv.). FAB-MS (NBA): from H_3L^1, *m*/*z* = 623 $[H_3L^1 + Zn - 2H]^+$; from HL^2, *m*/*z* = 651 $[HL^2 + Zn - 2H]^+$.

X-ray Crystallographic Study A single crystal of $[ZnFeL^1\{O_2P(OPh)_2\}_2]$ was mounted at 170 K on a Stoe IPDS diffractometer equipped with graphite-monochromated Mo-K_α radiation (λ = 0.71073 Å). $[ZnFe(C_{35}H_{33}N_4O_3)\{O_2P(OC_6H_5)_2\}_2]$ M = 1177.26, dark purple platelet (0.045 × 0.08 × 0.23 mm), monoclinic, space group $P2_1/c$, a = 19.338(4), b = 16.850(3), c = 16.851(3) Å, β = 99.12(3)°, U = 5421(2) Å^3, Z = 4, D_c = 1.44 g cm^{-3}, μ = 0.835 mm^{-1}. 43028 Reflections (10794 independent reflections) were collected and corrected for Lorentz and polarization effects but not for absorption. The structures were solved by direct methods and refined using the teXsan software package.[13] All non-hydrogen atoms were refined with anisotropic thermal parameters. Hydrogen atoms were generated in idealized positions, riding on the carrier atoms, with isotropic thermal parameters. Final cycle refinement, including 703 parameters, converged to $R(F) = 0.035$ and $Rw(F) = 0.028$ [for 6130 $F > 2\sigma(F)$] [s = 1.11, (Δ/σ_{max} = 0.07, $\Delta\rho_{max}$ = 0.36 e·Å^{-3}, $\Delta\rho_{min}$ = −0.26 e·Å^{-3}]. CCDC-179245 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; Fax: (internat.) +44-1223/336-033; E-mail: deposit@ccdc.cam.ac.uk].

---

[1] M. B. Twitchett, A. G. Sykes, *Eur. J. Inorg. Chem.* **1999**, 2105−2115.

[2] [2a] N. Sträter, T. Klabunde, P. Tucker, H. Witzel, B. Krebs, *Science* **1995**, *268*, 1489−1492. [2b] T. Klabunde, N. Sträter, R. Fröhlich, H. Witzel, B. Krebs, *J. Mol. Biol.* **1996**, *259*, 737−748.

[3] K. Schepers, B. Bremer, B. Krebs, G. Henkel, E. Althaus, B. Mosel, W. Müller-Warmuth, *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 531−533.

[4] A. S. Borovik, V. Papaefthymiou, L. F. Taylor, L. P. Anderson, L. Que Jr., *J. Am. Chem. Soc.* **1989**, *111*, 6183−6195.

[5] S. Albedyhl, M. T. Averbuch-Pouchot, C. Belle, B. Krebs, J. L. Pierre, E. Saint-Aman, S. Torelli, *Eur. J. Inorg. Chem.* **2001**, 1457−1464.

[6] S. K. Dutta, R. Werner, U. Flörke, S. Mohanta, K. K. Nanda, W. Haase, K. Nag, *Inorg. Chem.* **1996**, *35*, 2292−2300.

[7] J. Kamitani, R. Kawahara, M. Yashiro, M. Komiyama, *Chem. Lett.* **1998**, 1047−1048.

[8] M. Ghiladi, C. J. McKenzie, A. Meier, A. K. Powell, J. Ulstrup, S. Wocadlo, *J. Chem. Soc., Dalton Trans.* **1997**, 4011−4018.

[9] M. Suzuki, S. Fujinami, T. Hibino, H. Hori, Y. Maeda, A. Uehara, M. Suzuki, *Inorg. Chim. Acta* **1998**, *283*, 124−135.

[10] S. Albedhyl, D. Schnieders, A. Jancsó, T. Gajda, B. Krebs, *Eur. J. Inorg. Chem.* **2002**, 1400−1409.

[11] C. Belle, G. Gellon, C. Scheer, J. L. Pierre, *Tetrahedron. Lett.* **1994**, *35*, 7019−7022.

[12] C. Belle, J. L. Pierre, E. Saint-Aman, *New J. Chem.* **1998**, *22*, 1399−1402.

[13] J. L. Beck, L. A. McConachie, A. C. Summors, W. N. Arnold, J. de Jersey, B. Zerner, M. P. Hendrich, P. G. Debrunner, *Biochem. Biophys. Acta* **1986**, *869*, 61−68.

[14] C. Belle, I. Gautier-Luneau, J. L. Pierre, C. Scheer, E. Saint-Aman, *Inorg. Chem.* **1996**, *35*, 3706−3708.

[15] Molecular Structure Corporation (1995). teXsan, Single Crystal structure Analysis Software. Version 1.7.MSC, 3200 Research Forest Drive, The Woodlands, TX 77381, USA.

Received July 8, 2002
[I02369]