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# A new bipodal carboxy-bis(hydroxypyridinonate) ligand. Synthesis and complexation with copper(II), nickel(II) and zinc(II) in aqueous solution

M. Amélia Santos [a,*], Raquel Grazina [a], Péter Buglyó [b], Sofia Gama [a], Etelka Farkas [b]

[a] Centro de Química Estrutural, Complexo I, Instituto Superior Técnico, Av. Rovisco Pais, 1049-001 Lisbon, Portugal
[b] Department of Inorganic and Analytical Chemistry, University of Debrecen, H-4010 Debrecen, Hungary

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**Abstract**

A new tetradentate ligand having two 3-hydroxy-4-pyridinonate moieties and one carboxylate group appended to a cyclohexane backbone is reported, together with the corresponding bidentate hydroxypyridinonate pendant arm. The synthesis and characterization of these ligands, as well as their complexation properties towards the first transition series of metal ions M(II) (M = Cu, Ni, Zn) in aqueous solution, are described and discussed herein. The tetradentate ligand forms quite stable complexes with this series of metal ions and in solution, it is proved to be a much more effective chelator than the corresponding bidentate derivative. The stability constants of the complexes formed with this set of bivalent ions follow the Irving–Williams order Ni < Cu > Zn. The carboxylic group has no interaction with the metal centre, thus remaining free for potential interaction with biological ligands (e.g. proteins).


*Keywords:* Hydroxypyridinones; Hydroxypyridinonate complexes; Chelating agents; Bifunctional ligand

## 1. Introduction

The family of the hydroxypyridinones, specially the 3-hydroxy-4-pyridinone derivatives (3,4-HP), are ligands of great interest within the area of medical research. Firstly, their interest began with the biological activity of the naturally occurring mimosine and some derivatives, related with their interaction with some transition metal ions [1,2]. Lately, there was an increased interest on this family of compounds related with their potential clinical uses as iron-chelating agents [3–5]. Some of these compounds, such as the 1,2-dimethyl-3-hydroxy-4-pyridinone (commercially available as Deferiprone and referred in this paper as $HL^3$), have already been used in clinical trials [6]. Most of the studies recently described in the literature for this type of chelators are intended for pharmaceutical uses and have been addressed for the complexation with hard trivalent ions (mostly Al and Fe, because accumulation of these metal ions causes serious diseases [7,8], but also Ga and In, associated to radio-diagnose purposes [9]). Furthermore, some very recent studies have been focused on the inhibitory activity of 3,4-HP ligands on iron-containing metalloenzymes [10].

As part of an ongoing project on the development of chelators for bivalent metal ions ($Cu^{2+}$, $Zn^{2+}$, $Ni^{2+}$) with potential clinical use, we have decided to study a new ligand having two bidentate 3-hydroxy-4-pyridinonate (3,4-HP) and one carboxylate groups appended to a cyclic backbone. These 3,4-HP groups are expected to easily wrap these metal ions, providing conditions for the full metal ion coordination. On the other hand, besides the tetradenticity needed to improve the M(II) chelation effectiveness, this new ligand has the carboxylate group as an extra-function (free from the metal complexation), which can eventually improve the interaction (and molecular recognition) with specific biological subsets. In fact, to the presence of a carboxylate, as an extra functional group (besides the hydroxypyridi-

* Corresponding author. Fax: +351-1-846-4455
*E-mail address:* masantos@popsrv.ist.utl.pt (M. Amélia Santos).

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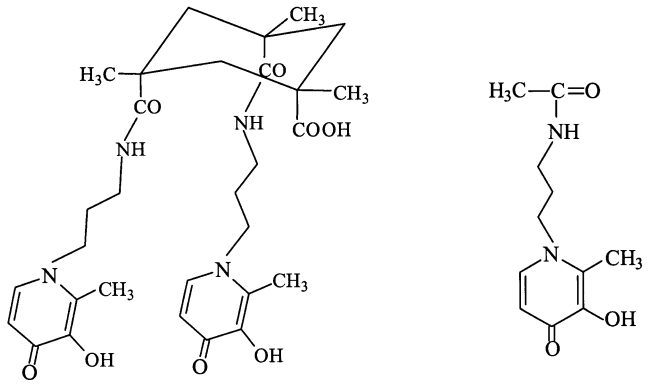
nones), has been recently proposed a special role on the increase of the bone uptake of $^{67}Ga$, thus, conferring to carboxyl–hydroxypyridonate derivatives a potential interest in bone tumour radiodiagnosis [11,12]. On the other hand, there is a large number of biological active MMP enzyme inhibitors which have a hydroxamate chelating group, as well as a carboxylate extra-functional group [13]. Some hydroxypyridinones, such as mimosine (β-*N*-(3-hydroxy-4-pyridone)-α-aminopropionic acid, herein named as $H_2L^4$), which has been also implicated in the inhibitory activity of various M(II)-containing enzymes (M = Cu, Zn) and other biological systems, possess also a 'free' carboxylate group [2].

Accordingly, this new ligand could be thought as a candidate for potential radiopharmaceutical, in relation to its chelation either with $^{64,67}Cu$, to be used in PET analysis [14], or with other $M^{2+}$ metal ions, for metalloenzyme inhibition.

In this paper, we report the study of a tetradentate bifunctional ligand, *cis*,*cis*-1,3-di-[1′-(3″-carboxyaminopropyl)-3′-hydroxy-2′-methyl-4′-pyridinone-yl]-5-carboxyl-1,3,5-trimethylcyclohexane, hereafter named as KEMP(NPrHP)$_2$ or $H_3L^1$ (Scheme 1). It is a bis(3-hydroxy-4-pyridinonate)-monocarboxylate ligand and so, herein, sometimes it is referred as a 'bis-chelator', in comparison with the corresponding 'mono-chelator' pendant unit, 1-(3′-methylcarboxyaminopropyl)-3-hydroxy-2-methyl-4-pyridinone), hereafter named AcNPrHP or $HL^2$, which was also studied. Each 3,4-HP unit is linked to an aminoalkylic chain, which acts both as a spacer and also a way of attachment to the cyclic backbone via its carboxylate groups. Besides the preparation and characterization of the ligands (acid–base and lipo-hydrophilic properties), their complexation ability towards the first transition series of divalent metal ions [Cu(II), Zn(II) and Ni(II)] as well as the corresponding speciation are described and discussed herein. The equilibrium studies were carried out in aqueous media and were based on potentiometric and spectrophotometric measurements. A brief molecular mechanics simulation was also carried out to get an insight on the ability of this new 'bis-chelator' to wrap this type of metal ions.




KEMP(NPrHP)$_2$, $H_3L^1$ AcNPrHP, $HL^2$

Scheme 1.

## 2. Results and discussion

### 2.1. Synthesis

The synthesis of this bis(hydroxypyridinonate)-carboxylate ligand involved a preliminary protection of one of the carboxylic functions of the starting material, the *cis*–*cis*-1,3,5-trimethylcyclohexane-1,3,5-tricarboxilic acid (KEMP acid), by its convertion into the intermediate cyclic anhydride [5-(chloroformyl)-*cis*,*cis*-1,3,5-trimethylcyclohexane-1,3-dicarboxilic anhydride], by standard methods [15]. This reaction takes advantage of the proximity between the carboxylic groups in the starting material and so it was performed with high yield. This intermediate was then coupled to 1-(3′-propylamine)-3-benzyloxy-4-pyridinone, to give the *O*-benzyl protected bis-hydroxypyridinone-mono-carboxylic acid derivative. To afford the final product, $H_3L^1$, the deprotection of the hydroxypyridinonate hydroxyl groups was performed by standard methods of hydrogenolysis [11]. The synthesis of the 'mono-chelator' compound, $HL^2$, involved a condensation of 1-(3′-propylamine)-3-benzyloxy-4-pyridinone with acethyl chloride.

### 2.2. Acid–base properties of the ligands

The bis- and the mono-hydroxypyridonate ligands, in their neutral form ($H_3L^1$ and $HL^2$), have only three and one labile protons, respectively, due to the carboxylic/phenolic groups, whereas, in their fully protonated form, they have the extra dissociable pyridinium protons. From the fitting analysis of potentiometric titration curves of the ligands (Fig. 1) with the SUPERQUAD program [16], five and two protonation constants were determined, respectively. Table 1 shows the calculated stepwise protonation constants together with the corresponding values for some representative 3-hydroxy-4-pyridinone analogues, such as 1,2-dimethyl-3-hydroxy-2-methyl-4-pyridinone ($HL^3$) [17] and mimosine ($H_2L^4$) [2]. Based on chemical evidences, the first two protonation constants of $H_3L^1$ ($\log K_a = 10.17$; 9.43) and the first one of $HL^2$ ($\log K_{a1} = 9.61$) are undoubtedly attributed to the phenolic groups. The three remaining $\log K_a$ values (4.70; 3.64; 2.96) of $H_3L^1$ are attributed to the carboxylic group and to the pyridinium groups. Although these three processes overlap each other, comparison between these stepwise protonation constants and the corresponding values for the pyridinium group of $HL^2$ (3.37) and other analogous compounds

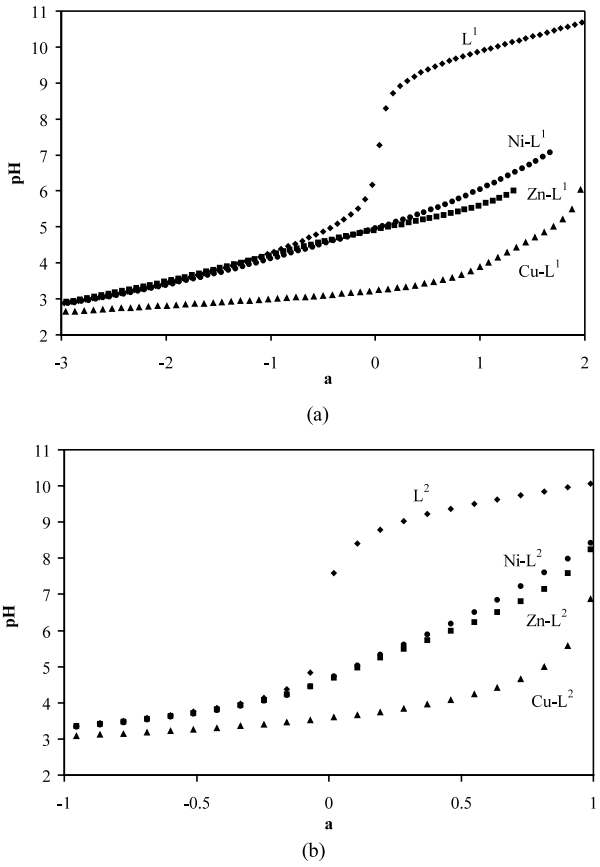



Fig. 1. Potentiometric titration curves for the ligands alone and in the presence of each M(II) metal ion (M = Cu, Ni, Zn): $H_3L^1$, at 1:1 metal to ligand molar ratio ($C_L = 2.9 \times 10^{-3}$ M, $I = 0.2$ M (KCl)) (a); $HL^2$, at 1:2 metal to ligand molar ratio. ($C_L = 1.6 \times 10^{-3}$ M, $I = 0.1$ M ($KNO_3$)) (b). $T = 25.0 \pm 0.1$ °C; $a$ = mols of base added per mol of ligand.

(see Table 1) suggests that the two lower values (3.64; 2.96) should be mainly attributed to the pyridinium groups. Besides, the higher value (4.70), attributed to the carboxylic group, is comparable to the log $K_a$ values of other tertiary carboxylic groups (ex. 2,2-dimethylpropanoic acid, log $K_a = 4.8$) [18]. Analysis of the speciation plots related to the calculated log $K_a$ values shows that the mono-anion $[H_2L^1]^-$ dominates over the pH range 5.5–8.5. Thus, this tetradentate ligand is expected to have a considerably higher hydrophilic character at the physiological conditions than the neutral bidentate derivative ($HL^2$).

### 2.3. Complexation studies

Complexation behaviour of these di- and mono-hydroxypyridinonate ligands ($H_3L^1$and $HL^2$) with the first transition series of metal ions [Cu(II), Ni(II) and Zn(II)] was studied by potentiometry and the interpretation of these results was aided by UV–Vis spectrophotometry. Precipitation was not observed in the samples containing some ligand excess but appeared at 1:1 ratio or at metal ion excess. The titrations were finished if precipitation occurred. Representative titration curves of these two ligands in the presence of each of those metal ions studied are shown in Fig. 1(a) ($H_3L^1$) and (b) ($HL^2$), for the 1:1 and in 2:1 ligand to metal molar ratios, respectively. Some species distribution diagrams for the complexation with the di-hydroxypyridonate ligand ($H_3L^1$) are presented in Fig. 2.

Analysis of the titration curves indicates that the metal complexation of both the ligands starts at a quite lower pH (approximately 2.5) with copper(II) than with nickel(II) and zinc(II) (approximately pH 4), thus, indicating a stronger interaction with copper(II). The experimental data presented good fittings for the complexation models having 1:1 and 1:2 stoichiometry for $H_3L^1$ and $HL^2$, respectively, with different protonation degrees. Comparison between the formation constants calculated for the complexes with $H_3L^1$ (Table 1) shows that log $\beta_{ML}$ is higher for Cu(II) than for Zn(II) and Ni(II) by a factor of 5.6 and 7.0 logarithmic units, respectively. Identical difference was found between the log $\beta_{ML_2^1}$ of the complexes of these metal ions with the monomeric derivatives, $HL^2$ and $HL^3$ (deferiprone) [17] (see Table 1).

Comparative analysis of the chelating abilities of $H_3L^1$ and the other analogues has to be made with care, mainly due to differences in the acid–base properties of the ligands and the stoichiometry of the complexes. Therefore, the pM values ($pM = -\log[M^{2+}]$) were also calculated for the different metal ions, at the physiological conditions (pH 7.4, Table 1) and for Cu(II), along a large range of pH (Fig. 3). A brief comparison of the complexing behaviour of the di-hydroxypyridinone and the mono-analogue suggests that the same binding sites (the 3-hydroxy-4-pyridinone portions) should be involved in the co-ordination of the ligands to each metal ion. In fact, for each metal ion, the profiles of the titration curves are very similar. The overall formation constants (log $\beta_{ML}$) calculated for the (1:1) complexes of this di-hydroxypyridinonate ligand with copper(II), nickel(II) and zinc(II) (17.42, 10.41, 11.78) are close to, albeit slightly lower than, the values (log $\beta_{ML_2}$) for the corresponding (1:2) complexes with the mono-hydroxypyridinonate ligands: $HL^2$, (18.99, 12.38, 13.70) and $HL^3$, (19.61, 12.13, 13.53) [17]. However, at conditions of 10-fold excess of the ligand and mM or μM concentrations, the pM values are higher for the dihydroxypyridinone, $H_3L^1$, (ex.: for $C_{Cu} = 10^{-4}$ M, pCu = 13.5; for $C_{Cu} = 10^{-6}$ M, pCu = 13.6) then the monohydroxypyridinone, $HL^2$, (ex.: for $C_{Cu} = 10^{-4}$ M, pCu = 12.4; for $C_{Cu} = 10^{-6}$ M, pCu = 10.4). Fig. 3 presents a graphical illustration of the higher copper binding ability of the bischelator KEMP(NPrHP)$_2$ ($H_3L^1$) over the monochelators ($HL^2$ and $HL^3$), along a range of pH above 4.

Table 1
Stepwise protonation constants of KEMP(NPrHP)$_2$ ($H_3L^1$) and relevant analogous; overall formation constants of their metal complexes (M = Cu, Ni, Zn) and the corresponding pM values * ($T = 25$ °C, $I = 0.2$ M (KCl))

| Ligand | $H_3L^1$ (KEMP2NPrHP) | $HL^2$ (AcNPrHP) [a] | $HL^3$ (3,4-DMHP) [b] | $H_2L^4$ (Mimosine) [c] |
|---|---|---|---|---|
| log *K*i | 10.17(2) | 9.61(2) | 9.86 | 8.86 |
| | 9.43(2) | 3.57(3) | 3.70 | 7.00 |
| | 4.70(2) | | | 2.62 |
| | 3.64(1) | | | 1.1 |
| | 2.96(3) | | | |
| log $\beta_{Cu_pH_qL_r}$ | (1, 1, 1) = 22.05(2) | (1, 0, 1) = 10.29(1) | (1, 0, 1) = 10.62 | (1, 1, 1) = 16.36 |
| | (1, 0, 1) = 17.42(2) | (1, 0, 2) = 18.99(2) | (1, 0, 2) = 19.61 | (1, 0, 1) = 9.48 |
| | | | | (1, 1, 2) = 24.40 |
| | | | | (1, 0, 2) = 16.81 |
| pCu | 13.6 | 10.4 | 10.5 | 9.9 |
| log $\beta_{Ni_pH_qL_r}$ | (1, 1, 1) = 17.07(1) | (1, 0, 1) = 6.99(5) | (1, 0, 1) = 6.92 | |
| | (1, 0, 1) = 10.41(2) | (1, 0, 2) = 12.38(9) | (1, 0, 2) = 12.13 | |
| pNi | 7.9 | 6.2 | 6.3 | |
| log $\beta_{Zn_pH_qL_r}$ | (1, 1, 1) = 17.39(1) | (1, 0, 1) = 7.19(9) | (1, 0, 1) = 7.19 | (1, 1, 1) = 13.84 |
| | (1, 0, 1) = 11.78(1) | (1, 0, 2) = 13.70(9) | (1, 0, 2) = 13.53 | (1, 0, 1) = 6.50 |
| | | | | (1, 1, 2) = 19.76 |
| | | | | (1, 0, 2) = 12.27 |
| pZn | 7.9 | 6.3 | 6.2 | 6.4 |

* The equilibrium process to which the overall stability constant (log $\beta$) relates is: $pM + qH + rL \rightleftarrows M_pH_qL_r$; $\beta = [M_pH_qL_r]/[M]^p[H]^q[L]^r$; the (p, q, r) symbolism means a species with stoichiometry ($M_pH_qL_r$); $pM = -\log[M^{2+}]$ with $C_L/C_M = 10$, $C_M = 10^{-6}$ M) and at pH 7.4.
[a] $I = 0.1$ M, $KNO_3$.
[b] According to Ref. [17].
[c] According to Ref. [2].

The stability constants of the di-hydroxypyridone ($H_3L^1$) complexes with this set of bivalent metal ions seem to follow the proposed Irving–Williams order Ni < Cu > Zn [19], as it happens with hydroxamate complexes [20].

Regarding the type of binding modes in $MHL^1$ species, two different possibilities can be admitted. As a first possibility, two hydroxypyridinonate groups could be involved in the co-ordination to the metal ion, leaving the dissociable proton in the carboxylic group. Such a co-ordination mode could be favourable if the complex species was formed when the carboxylic moiety is protonated (below pH 4, as happens with the copper complexes, see Figs. 1 and 2(a)). As a second possibility, one of the hydroxypyridinonate groups is in the co-ordinated form while the other one is in protonated form; this complex would be favoured in the pH-range where the carboxylic moiety is already deprotonated (above pH 4, as happens for the nickel and zinc complexes, see Figs. 1 and 2(b)).

In order to get some insight into the co-ordination modes involved in the copper(II) complexes with the di-hydroxypyridonate ligand, UV–Vis absorption spectra of solutions containing this metal ion in the presence of $H_3L^1$ or $HL^2$ were registered at different pH (Fig. 4), for comparison purpose. Analysis of Fig. 4 confirms that, in both the systems, complexation has already started at approximately pH 2.5, in accordance with the species distribution diagram (Fig. 2(a)). At pH 3.5, the samples containing the copper(II)–$H_3L^1$complexes exhibit a *d*–*d* transition band centred at 680 nm ($\varepsilon = 43$ $M^{-1}$ $cm^{-1}$) and a charge transfer transition ($\lambda_{max} = 377$ nm, $\varepsilon = 250$ $M^{-1}$ $cm^{-1}$). From the species distribution diagram, it can be mostly assigned to the $CuL^1H$ species. The similarity between the *d*–*d* transition band of this complex and the corresponding bands for the 1:2 complexes with $HL^2$ ($\lambda_{max} = 675$ nm, $\varepsilon = 59$ $M^{-1}$ $cm^{-1}$, at pH 5.56), $HL^3$ ($\lambda_{max} = 692$ nm, $\varepsilon = 31$ $M^{-1}$ $cm^{-1}$) [17] and $H_2L^4$ ($\lambda_{max} = 690$ nm, $\varepsilon = 33$ $M^{-1}$ $cm^{-1}$) [2] gives support to the assumption made on the binding mode through the hydroxypyridinonate moieties. For higher pH values ($3.5 < pH < 5.3$), there is a small blue shift of $\lambda_{max}$ to 665 nm ($\varepsilon = 51$ $M^{-1}$ $cm^{-1}$) which may be associated with some formation of the $CuL^1$ species and some strengthening of metal–ligand bond in these species.

Above pH 5.3, more than 80% of the copper complex is in the neutral form ($CuL^1$) (see Fig. 2(b)) and precipitation starts in our experimental conditions.

Concerning the nickel(II) complexes, at pH 6.7, the UV–Vis spectra presented a charge transfer transition ($\lambda_{max} = 392$ nm, $\varepsilon = 66$ $M^{-1}$ $cm^{-1}$) and a very week band in the *d*–*d* region ($\lambda_{max} = 673$ nm, $\varepsilon = 15$ $M^{-1}$ $cm^{-1}$). This should correspond to the neutral bis-chelated species (NiL, approximately 50%) which started to precipitate with increasing concentration (at pH $\geq 7$). According to the spectra, octahedral or pseudo-octahedral geometry around the metal ion is suggested. Since only four coordination sites are occupied by two hydroxypyridinonate moieties, probably

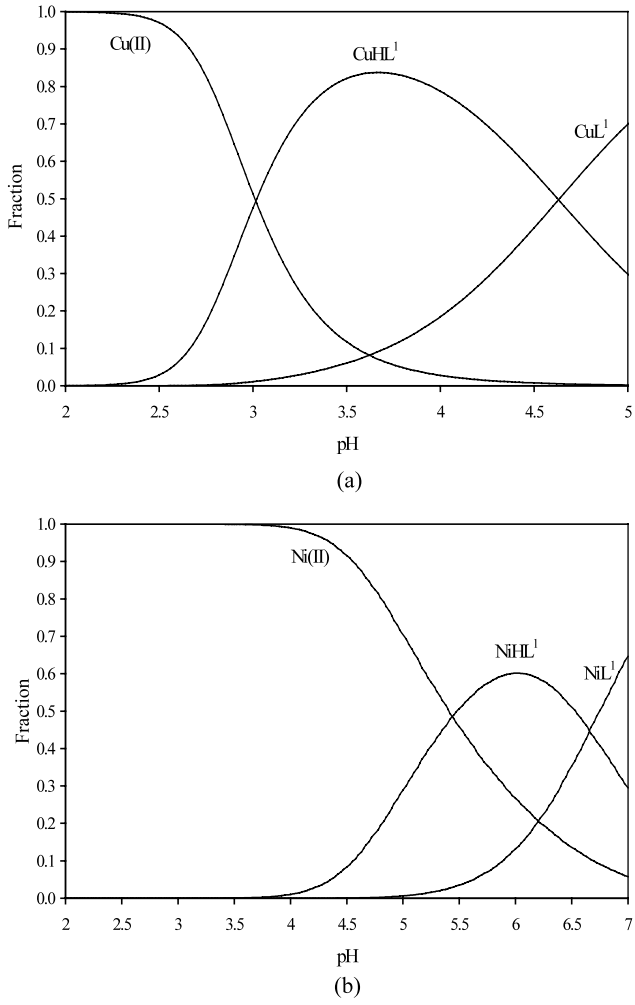



Fig. 2. Concentration distribution curves calculated for the Cu–$H_3L^1$ complexes (a) and Ni–$H_3L^1$ complexes (b), at 1:1 metal to ligand ratio and $C_L = 2.9 \times 10^{-3}$ M.

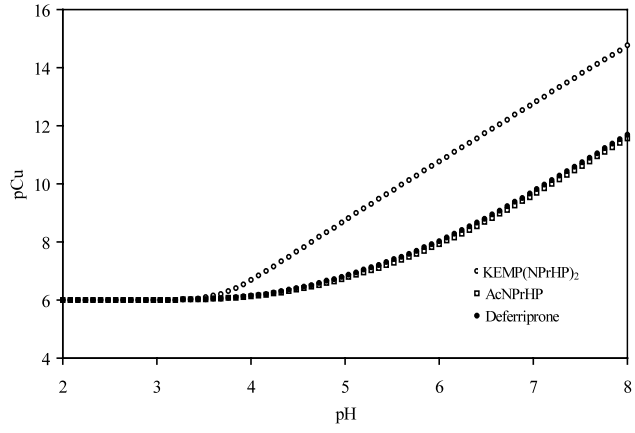



Fig. 3. Copper complexation strength for $H_3L^1$ ($KEMP(NPrHP)_2$), $HL^2$ (AcNPrHP) and $HL^3$ (Deferiprone), reported as pCu vs. pH ($pCu = -\log[Cu^{2+}]$ with $C_L/C_{Cu} = 10$ and $C_{Cu} = 10^{-6}$ M).

two water molecules are also involved to complete the co-ordination around the metal centre. The involvement

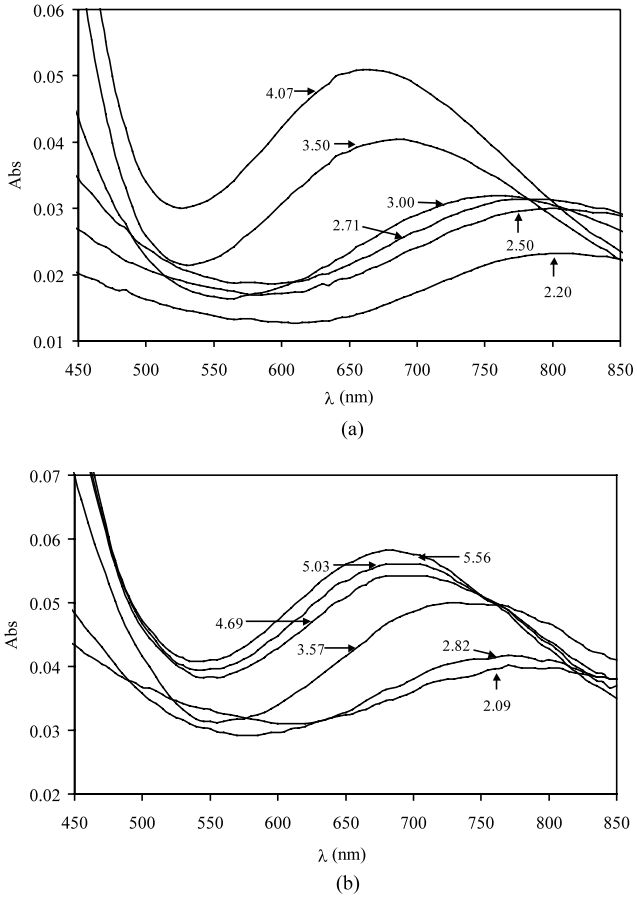



Fig. 4. Absorption spectra registered at various pH values for: the Cu(II)–$H_3L^1$ system ($C_L = 1.5 \times 10^{-3}$ M, $C_L/C_M = 1.5$) (a); the Cu(II)–$HL^2$ system ($C_L = 2.0 \times 10^{-3}$ M, $C_L/C_M = 2$).

of the carboxylate group in the coordination can hardly be assumed, due to steric demands.

Regarding the zinc(II) complexes, a brief comparative analysis of the $^1H$ NMR spectra was performed for complex and ligand solutions in the pH range 4–6. These results showed that the highest deviations in the chemical shifts, $\Delta\delta = -(0.2–0.4)$ ppm, were observed for the two hydroxypyridinone ring protons, thus suggesting the involvement of the hydroxypyridinonate moieties in the co-ordination to the metal centre.

Furthermore, as stated above, the hydroxypyridonate coordination in the metal complexes is also supported by the similarity between the potentiometric titration profiles and also between the overall formation constants obtained for the 1:1 complexes and the 1:2 complexes with $H_3L^1$ and $HL^2$, respectively.

Finaly, aimed at giving some contribution to the interpretation of the complexation behaviour of the dihydroxypyridonate ligand, a brief molecular modelling calculation was performed for this ligand (using MOPAC program included in the INSIGHT package [21]) and the Cu–$L^1$ complex (using molecular mechanics, with the CERIUS2 [22] program and the default force

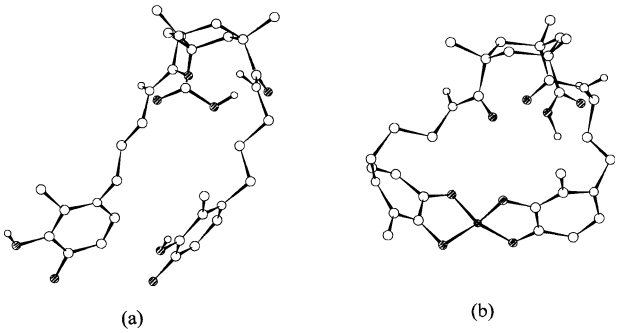



Fig. 5. Structure diagrams calculated for: the ligand $H_3L^1$, KEMP(NPrHP)$_2$, (a) and the $CuL^1$ complex (b). The metal ions are doubly hatched; the oxygen atoms are hatched; hydrogen atoms bonded to carbon atoms are omitted.

fields [23]). The simulated structure of the ligand (Fig. 5(a)) suggested a *chair* conformation for the cyclohexane backbone with the alkylamide-hydroxypyridinonate and the carboxylate pendant arms in axial position, thus, pre-orientated to wrap the metal ion. That conformation is apparently supported by some hydrogen-bond interaction between the neighbouring pendant arms. Concerning the Cu–$L^1$ complex, a distorted square-planar coordination geometry seems probable, with no significant interaction between the carboxylate group and the metal ion, due to steric demands. The coordination of the ligand towards the Cu(II) ion requires the disruption of the hydrogen-bond network found in the ligand, although the *chair* conformation was kept in the minimum energy conformer (Fig. 5(b)).

### 2.4. Distribution coefficient

The distribution coefficient of the free ligand between the aqueous phase (buffered at pH 7.4 with Tris) and octanol showed that, at physiological pH, the 'dimeric' ligand ($\log D = -1.96 \pm 0.02$) is much more hydrophilic than the corresponding monomeric species ($\log D = -1.04 \pm 0.02$). This is due to the fact that, at this pH, the 'dimeric' ligand is mono-charged $[H_2L^1]^-$ as a result of the dissociation of the carboxylic proton. Even so, it is much less hydrophilic than other (carboxyalkyl)hydroxypyridinones [$\log D = -(2.8–2.9)$] [12] due to some hydrophobicity induced by the higher ratio between the number of hydroxypyridinonate and carboxylate groups (2:1 instead of 1:1).

## 3. Conclusions

This new heteropodant bis-hydroxypyridonate ligand forms quite stable 1:1 complexes with the bivalent metal ions of the first transition series. The metal co-ordination environment is dominated by the hydroxypyridinonate moieties, thus leaving the extra carboxylic group for further eventual interaction with biological ligands (e.g. proteins). It has proved a good lipo-hydrophilic balance. So, important biological roles may be admitted for this ligand, probably as an enzymatic inhibitor, related to its affinity for Ni(II) and Zn(II), or as a good chelating agent for radio-diagnosis (PET) due to its high affinity for $^{64,67}$Cu(II).

## 4. Experimental

### 4.1. Chemicals

Analytical grade reagents were used as supplied. Whenever necessary, solvents were dried according to standard methods [24]. All the chemical reactions were TLC controlled.

#### 4.1.1. Synthesis of the ligand, $H_3L^1$ (or KEMP(NPrHP)$_2$) (**1**)

*4.1.1.1. 1-(3′-Aminopropyl)-3-benzyloxy-2-methyl-4-pyridinone (**3**).* 3-Benzyloxy-2-methyl-4-pyrone (2.3 g, 10.7 mmol), obtained according to previously described [11], and 1,3-diaminopropane (1.1 ml, 13.2 mmol) were left refluxing in water (30 ml) for 6 h. Evaporation of water and excess of 1,3-diaminopropane in vacuum gave an oil which was then dissolved in MeOH. Acidification of that solution until pH 2 with HCl saturated MeOH gave a white precipitate which was recrystallized from dry MeOH–MeCN to give the pure product ($\eta = 40\%$). M.p. 186–189 °C; IR (cm$^{-1}$, KBr) 1630 ($\nu_{C=O}$). $^1$H NMR ($CDCl_3$) 8.14 (1H, d, 6-HPy), 7.42 (5H, s, Ph), 7.11 (1H, d, 5-HPy), 5.13 (2H, s, $CH_2$Ph), 4.31 (2H, t, $CH_2$N–Pyr), 3.03 (2H, t, $CH_2$N), 2.36 (2H, t, $CH_2CH_2$-$CH_2$), 2.12 (3H, s, Me). *m/z* (FAB) 273 ($M+1$).

*4.1.1.2. 5-(Ch1oroformyl)-cis,cis-1,3,5-trimethylcyclohexane-1,3-dicarboxilic anhydride (**4**).* To a solution of 1,3,5-trimethyl-1,3,5-cyclohexane-tricarboxylic acid (1.95 g, 7.4 mmol) in dry $CH_2Cl_2$ (50 ml), oxalyl chloride (0.8 ml) was added as well as one drop of dry dimethylformamide (DMF). The mixture was left refluxing under dry nitrogen atmosphere for 4 h. Excess of oxalyl chloride was removed by rota-evaporation. Two 10 ml batches of dry $CHCl_3$ were subsequently added to the residue and then removed by vacuum distillation, giving a white solid. Recrystallisation from dry toluene gave the pure product ($\eta = 85\%$). M.p. 254–258 °C (255–260 °C) [15]. IR (KBr): 1790 cm$^{-1}$ ($\nu_{C=O}$). $^1$H NMR ($C_6D_6$) $\delta$: 0.11, 0.77 (2H, 2 × d, $J =$ 14.1 Hz, $CH_2$Cy); 0.32, 2.33 (4H, 2 × d, $J = 14.1$ Hz, $CH_2$Cy); 0.86 (6H, s, $CH_3$), 0.59 (3H, s, $CH_3$). *m/z* (FAB): 260 ($M+1$).

*4.1.1.3. Cis,cis-1,3-di-(1′-(3″-carboxyaminopropyl)-3′-benzyloxy-2′-methyl-4′-pyridinone-yl)-5-carboxyl-1,3,5-*

*trimethyl-cyclohexane (**5**)*. To a solution of **3** (1.7 g; 6.25 mmol) and $Et_3N$ (2.6 ml, 18.8 mmol) in dry $CH_2Cl_2$ (50 ml), under a nitrogen atmosphere and in ice-water bath, a freshly prepared solution of **4** (0.572 g, 2.21 mmol) in dry $CH_2Cl_2$ (15 ml) was drop-wise added. The reaction mixture was then stirred at room temperature (r.t.) for 6 h. The solvent was removed under reduced pressure and the solid residue was purified by column chromatography on silica gel with $CH_2Cl_2$–MeOH (30/8) as the eluent. The isolated product was recrystallized from dry MeOH–MeCN ($\eta = 48\%$). M.p. 189–191 °C. IR ($cm^{-1}$, KBr): 2374 ($\nu_{NH+}$); 1626, 1558 ($\nu_{C=O}$). $^1H$ NMR ($CD_3OD$) $\delta$: 7.74 (2H, d, 6-HPy), 7.30 (10H, s, Ph), 6.31 (2H, d, 5-HPy), 5.01 (4H, s, $CH_2Ph$), 3.96 (4H, t, $CH_2NPy$); 3.07, 2.82 (4H, 2 × m, $CH_2NCO$), 2.72 (4H, d, $CH_2Cy$), 2.12 (6H, s, Me–Py), 1.72 (4H, t, $CH_2CH_2CH_2$), 1.17 (3H, s, $CH_3$), 1.08 (6H, s, $CH_3Cy$), 0.85 (2H, d, $CH_2Cy$). *m/z* (FAB): 767 ($M+1$).

*4.1.1.4. cis,cis-1,3-Di-(1′-(3″-carboxyaminopropyl)-3′-hydroxy-2′-methyl-4′-pyridinonyl)-5-carboxyl-1,3,5-trimethyl-cyclohexane, $H_3L^1$ (**1**)*. To a solution of **5** (0.2 g, 0.26 mmol) in dry MeOH (50 ml) was added 10% Pd/C (0.05 g) and the mixture was stirred under $H_2$ (1 atm) for 5 h at r.t. After filtration of the solid residue, the solvent was evaporated under reduced pressure and the product was obtained as a white powder, which was then recrystallized from MeOH–MeCN ($\eta = 80\%$). M.p. 180–182 °C. IR ($cm^{-1}$, KBr): 3285 ($\nu_{OH}$), 2361 ($\nu_{NH+}$); 1629, 1568 ($\nu_{C=O}$). $^1H$ NMR (MeOD) $\delta$: 7.65 (2H, d, 6-HPy), 6.31 (2H, d, 5-HPy), 3.95 (4H, t, $CH_2NPy$), 3.04, 2.86 (2 × 2H, 2 × m, $CH_2NCO$), 2.72 (4H, d, $CH_2Cy$), 2.343 (6H, s, Me–Py), 1.77 (4H, t, $CH_2CH_2CH_2$), 1.17 (3H, s, $CH_3Cy$), 1.08 (6H, s, $CH_3Cy$), 0.85 (2H, d, $CH_2Cy$). *m/z* (FAB): 587 ($M+1$). *Anal.* Calc. for $C_{30}N_4H_{42}O_8 \cdot 3H_2O$: C, 56.27; H, 7.50; N, 8.75. Found: C, 56.6; H, 7.58; N, 8.42%.

*4.1.2. Synthesis of the ligand $HL^2$ (or AcNPrHP) (**2**)*

*4.1.2.1. 1-(3′-Methylcarboxyaminopropyl)-3-benzyloxy-2-methyl-4-pyridinone (**6**)*. To a solution of 1-(3′-aminopropyl)-3-benzyloxy-2-methyl-4-pyridinone (0.46 g; 1.69 mmol) and *N*-methylmorpholine (0.17 g; 1.69 mmol) in dry MeOH (15 ml), cooled in a ice-water bath, was dropwise added a solution of acethylchloride (0.17 g; 2.20 mmol) in dry THF (10 ml). After the addition, the reactional mixture was left under stirring at r.t. for 3 h. The *N*-methylmorpholinium salt, formed during the reaction, was filtered off and the solvents were removed under reduced pressure. The obtained product was than purified by flash column chromatography on silicagel with $CH_2Cl_2$–MeOH (30:7) as the eluent. The pure product was isolated as a pale oil ($\eta =$ 47%). $^1H$ NMR ($CD_3OD$) $\delta$: 7.63 (1H, d, 6-HPy), 7.26 (5H, s, Ph), 6.39 (1H, d, 5-HPy), 5.00 (2H, s, $CH_2Bz$), 3.90 (2H, t, $CH_2NPy$), 3.10 (2H, t, $CH_2NH$), 2.09 (3H, s, $CH_3Py$), 1.86 (3H, s, $CH_3C{=}O$), 1.76 (2H, q, $CH_2CH_2CH_2$). *m/z* (FAB): 315 ($M+1$).

*4.1.2.2. 1-(3′-Methylcarboxyaminopropyl)-3-hydroxy-2-methyl-4-pyridinone, $HL^2$ (**2**)*. To a solution of **6** (0.25 g; 0.80 mmol) in dry MeOH (10 ml) was added 10% Pd/C (0.08 g) and the mixture was stirred under $H_2$ (1.4 atm) for 4 h at r.t. After the solid residue filtration, the solvent was evaporated under reduced pressure and product was obtained as a pale solid. Recrystallization from dry MeOH–MeCN afforded the pure product as pale crystals ($\eta = 84\%$). M.p. = 135–136 °C. $^1H$ NMR ($CD_3OD$) $\delta$: 7.53 (1H, d, 6-HPy), 6.30 (1H, d, 5-HPy), 3.98 (2H, t, $CH_2NPy$), 3.14 (2H, t, $CH_2NH$), 2.34 (3H, s, $CH_3Py$), 1.85 (3H, s, $CH_3C{=}O$), 1.79 (2H, q, $CH_2CH_2CH_2$). *m/z* (FAB): 225 ($M+1$). *Anal.* Calc. for $C_{11}N_2H_{16}O_3 \cdot 1.3H_2O$: C, 53.34; H, 7.57; N, 11.31. Found: C, 53.59; H, 7.69; N, 10.82%.

### 4.2. Potentiometric measurements

The potentiometric measurements were performed at 25 ± 0.1 °C with a MOLSPIN pH-Meter equipped with a Metrohm combined electrode and Mol-ACS microburette controlled by computer. The electrode system was calibrated by the method of Irving et al. [25], so that the pH-meter readings could be converted into hydrogen ion concentrations. Atmospheric $CO_2$ was excluded from the system with a purging stream of argon gas. The ligand concentration in the sample was $2.9 \times 10^{-3}$ M ($H_3L^1$) and $1.6 \times 10^{-3}$ M ($HL^2$) with ionic strength of 0.2 M (KCl) and 0.1 M ($KNO_3$), respectively. The concentration of the ligand stock solutions together with the protonation constants was determined from the potentiometric data by the use of the SUPERQUAD computer program [16].

The complexation studies were performed for each metal ion by potentiometric titrations, with 1:1 and 1:2 metal-ion:ligand ratios. The metal ions were provided from metal-ion stock solutions of the corresponding chloride or nitrate salts. The stability constants were determined using the computer program PSEQUAD [26].

The selection of the equilibrium models was based on the critical analysis of the weighted residuals and the statistical parameters ($\chi^2$ and $\sigma$ for the SUPERQUAD [27] and the fitting parameter for the PSEQUAD) as well as on graphical comparisons between the experimental and simulated potentiometric curves.. The fitting parameters obtained for the equilibrium models of each ligand–metal ion system were less than $2 \times 10^{-3}$.

### 4.3. Spectrophotometric measurements

All spectra were measured on a HP 8453 and a Lambda 9 Perkin–Elmer spectrophotometer at 25 °C

and at constant ionic strengths. Solutions of the metal complexes were generated in situ by addition of a standard metal ion solution to the ligand solution. The pH measurements were carried out using a 420A Orion pH-meter, equipped with an Orion 91-03 glass calomel combination electrode.

### 4.4. Partition coefficients

Octanol–water partition coefficients of the ligands were determined at physiological pH, at approximately 25 °C, as described previously [11]. Equal volumes (3 ml) of $2 \times 10^{-4}$ M ligand solutions in octanol and water ($2 \times 10^{-3}$ M tris buffered solution, pH 7.4) were placed in a 20 ml screw-cap vial. The mixture was stirred for 18 h, allowed to stand for 3 h and then poured into an extraction funnel to separate the two phases. After adequate dilution of the samples, the species concentration was evaluated by UV spectrophotometry (using the 285–295 nm $\lambda_{max}$ bands). The partition coefficients, $D$ (or $\log D$), were obtained as the ratio of the ligand concentrations in the organic phase to that in the aqueous phase. Both the solvents used in all determinations (octanol and buffered water solution) were previously saturated in each other, by stirring the mixture for 18 h.

### 4.5. Other measurements

The $^1$H NMR spectra were recorded on a Varian Unity 300 spectrometer at 25 °C. Chemical shifts are reported in ppm ($\delta$) from internal reference tetramethylsilane (TMS) in organic solvents and sodium 3-(trimethylsilyl)-[2,2,3,3-$^2H_4$]propionate in $D_2O$ solutions (DSS). The following abbreviations are used: d, duplet; s, singlet; t, triplet. Melting temperatures were measured with a Leica Galen III hot stage apparatus and are uncorrected. Elemental analysis was performed on a Fisons EA1108 CHNF/O instrument. Mass spectra were recorded on a VG TRIO-2000 GC/MS instrument.

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