Formation of very stable and selective Cu(II) complexes with a non-macrocyclic ligand: can basicity rival pre-organization?†

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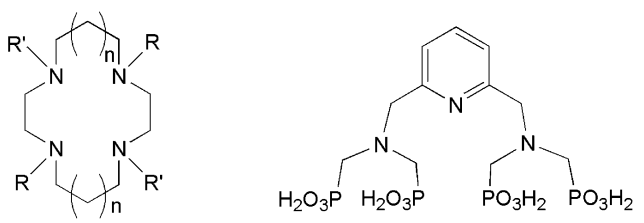
The synthesis of ligand **L** based on a 2,6-bis[(*N*,*N*-bis(methylene phosphonic acid)aminomethyl] pyridine scaffold is described. Potentiometry combined with UV-Vis absorption spectrophotometric titrations were used to determine the protonation constants of the ligand and the stability constants of its corresponding Cu(II), Ni(II), Zn(II) and Ga(III) cations (0.1 M $NaClO_4$, 25.0 °C). The physico–chemical approach revealed very large stability constants for Cu(II) complexation ($\log K_{CuL}$ = 22.71(7)) reflected in a very high pCu^{II} value of ~ 15.5 (pH = 7.4, $[L]_{tot} = 10^{-5}$ M, $[Cu]_{tot} = 10^{-6}$ M), close to those measured for the strong methylphosphonate functionalized cyclen chelators. Based on a literature survey, a correlation is proposed between the p*K* values of branched polyamine ligands and their stability constants for Cu(II) complexation, allowing for an estimation of the latter on the basis of the protonation constants of **L**. Ligand **L** was also shown to be very selective towards Cu(II) compared to the other cations studied ($\Delta\log K > 4$). UV-Vis spectroscopy and kinetic measurements indicated that the formation of the cupric complexes with **L** is very fast, which, in combination with all other properties, makes it an excellent non-cyclic target for Cu(II) radiopharmaceutical within the frame of ^{64}Cu positron emission tomography imaging and radiotherapy.

Introduction

Copper cation plays a major role in many biological systems and misregulation of its homeostasis can result in severe illness.[1] For instance, there is significant evidence to suggest that Cu(II), among others (*e.g.* Zn(II) and Fe(III)), is directly involved in the pathogenesis of Alzheimer's disease.[2] Indeed, β-Amyloid peptides (Aβ) display high affinities for Cu(II) (K_D ~ 1 μM),[3] which mediates its precipitation as insoluble and toxic aggregates.[4] These metal ions being highly concentrated in the neocortex of AD patients,[5] the Cu(II) binding by Aβ might cause the production of reactive oxygen species (ROS), which are also associated to neurodegeneration.[5,6] It was shown that Zn(II)- or Cu(II)-induced Aβ precipitation is reversed by treating the aggregates with exogenous ligands,[7] justifying the search for biocompatible Cu(II) chelators as potential therapeutic agents.[8]

Concomitantly, the increasing number of available research cyclotrons worldwide, together with the advances in the production of ^{64}Cu from ^{64}Ni,[9] recently led to a revival of the interest in ^{64}Cu and ^{67}Cu chelation and in its application to positron emission tomography (PET) imaging and radiotherapy.[10] For this purpose, various bifunctional chelators (BFCs)[11] or copper(II) complexing reagents[12] have been designed to firmly and selectively hold ^{64}Cu and to deliver it to the biological targets.

Up until now, most of the BFCs for Cu(II) coordination[11] were concerned with cyclen and cyclam based structures and their branched or cross-bridged analogues (R ≠ H in Chart 1).[11] Although, they were undoubtedly the best candidates regarding their *in vitro* stability, they sometime present drawbacks such as tedious synthetic protocols (especially for the introduction of the labeling functions for grafting on biomaterials), slow kinetics for Cu(II) complexation often due to a "proton sponge" effect,[12] or weak *in vivo* stabilities.[13]



n = 0, R = H, cyclen
n = 1, R = H, cyclam

LH_8

Chart 1 Chemical structures of cyclen, cyclam and LH_8

In our quest to fulfil the criteria for ^{64}Cu complexation: *i.e.* (i) strong Cu(II) complexation; (ii) selectivity towards Zn(II), Ni(II) and endogenous cations; (iii) fast kinetic of complexation at room temperature and (iv) stability towards reduction to Cu(I), we developed the synthesis of ligand **L**, a 2,6-diaminomethylpyridine functionalized with four methanephosphonic acid functions.

Results and discussion

Synthesis of the ligand

The synthetic protocol is summarized in Scheme 1. Starting from the commercially available 2,6-dihydroxymethyl pyridine,

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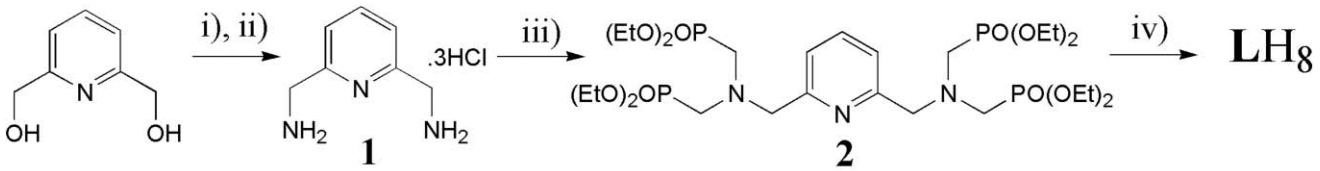
† Electronic supplementary information (ESI) available: ^{1}H and ^{13}C-NMR spectra of **L**; list of ligands used for Fig. 4 and corresponding references of the literature. See DOI: 10.1039/c0dt00453g



Scheme 1 Synthesis of ligand LH_8 (i) PBr_3, DMF, 84%. (ii) HMTA, $CHCl_3$, 80 °C, 3 h; conc. HCl, MeOH, reflux, 17 h, quant.[14] (iii) EtOH, NaOH, reflux, 1 h; $HPO(OEt)_2$, 37% formaldehyde in H_2O, 0 °C; 100 °C, 60%. (iv) TMSBr, CH_2Cl_2; MeOH, 83%.

2,6-dibromomethylpyridine was obtained by a bromination with PBr_3. Following a literature procedure, a Delepine-type reaction afforded the 2,6-diaminomethylpyridine **1**,[14] which was further converted to the tetramethylphosphonic ethyl ester **2** by reaction with formaldehyde and diethylphosphite. The phosphonic acid functions were recovered by hydrolysis of the phosphonic esters with TMSBr followed by methanolysis to afford LH_8 as its hydrated hydrobromic salt.

Physico–chemical studies. Using a combination of pH-metric and UV-Vis absorption spectrophotometric *vs.* pH titrations, it was possible to determine the protonation constants (K_{LHx}) of $\mathbf{L}^{8-}$ (*i.e.* the fully deprotonated ligand) and the corresponding stability constants (K_{MLHx}) for the formation of the metal complexes with Cu(II), Zn(II), Ni(II) and Ga(III) (water; I = 0.1 M $NaClO_4$; T = 25.0(2) °C). All the thermodynamic data are gathered in Table 1, together with some relevant data obtained for ligand **L′**, an analogue of **L** containing carboxylic acids instead of the phosphonic functions in **L**, and **DO2P**, one of the most efficient polyaminomethanephosphonic ligand for Cu(II) complexation, to the best of our knowledge.[15]

Within the pH range studied (2.5 < pH < 12), ligand **L** displayed two basic protonation constants above pH 10 (Table 1), which were unambiguously assigned, by analogy with **L′**, to the protonation of the two tertiary amine functions. The $\log K_{LH}$ and $\log K_{LH2}$ values are more than two orders of magnitude higher than the corresponding ones measured for the analogue **L′**, which can be explained by the stronger repulsions of the negative charges of the $-PO_3^{2-}$ moieties with respect to the $-CO_2^-$ units, which prevails over the electron withdrawing effect.[18,19] Markedly different hydrogen bonding between the protonated ammonium units and the negatively charged phosphonates or carboxylate moieties can also largely account for the very different acido–basic properties.[20] Intricate hydrogen bond networks involving the pyridine chromophore cannot be, indeed, excluded since a spectrophotometric titration *vs.* pH of the free ligand **L** indicated significant variations of the pyridine-centered transitions (hypochromic shift), particularly when the two tertiary amines and the two first methanephosphonates are protonated.

Table 1 Successive protonation constants ($\log K_{LHx}$), formal stability constants ($\log K_{ML}$) and pCu^{II} values for ligands **L**,[a] **L′**[b] and **DO2P**[c]

	L[a]	**L′**[b]	**DO2P**[c]
$\log K_{LH}$	11.21(2)	8.95	12.80(8)
$\log K_{LH2}$	10.29(2)	7.85	10.92(2)
$\log K_{LH3}$	8.04(4)	3.38	8.47(2)
$\log K_{LH4}$	6.49(6)	2.48	6.39(2)
$\log K_{LH5}$	5.53(8)		
$\log K_{LH6}$	4.19(9)		
$\log K_{CuL}$	22.71(7)	15.69(2)	28.7
$\log K_{NiL}$	16.50(3)		
$\log K_{ZnL}$	17.84(4)	15.84(2)	21.2(3)
$\log K_{GaL}$	16.31(9)		
pCu^{II}[d]	14.4[d]/15.5[e]	12.7[d]/12.7[e]	17.8[d]

[a] Solvent: water; I = 0.1 M ($NaClO_4$); T = 25.0(2) °C. Errors = 3σ with σ = standard deviation. $K_{LHx} = [LH_x]/[[LH_{x-1}][H]$ and $K_{ML} = [ML]/[L][M]$. Charges have been omitted for the sake of clarity. $\log K_{Cu(OH)} = -6.29$ and $\log K_{Cu(OH)2} = -13.1$; $\log K_{Zn(OH)} = -7.89$ and $\log K_{Zn(OH)2} = -14.92$; $\log K_{Ni(OH)} = -8.1$ and $\log K_{Ni(OH)2} = -16.87$ (16a); $\log K_{Ga(OH)} = -2.65$ (16b). [b] **L′** = 2,6-*Bis*[*bis*((carboxymethyl)amino)methyl]pyridine (17); solvent: water; T = 25 °C. [c] **DO2P** = 1,4,7,10-Tetraazacyclododecane-1,7-bis(methane phosphonic acid) (15); solvent: water; I = 0.1 M (KCl); T = 25 °C. [d] $pCu^{II} = -\log[Cu(II)]_{free}$ for $[Cu(II)]_{tot} = 10^{-6}$ M, $[L]_{tot} = 10^{-5}$ M, pH = 7.4 and calculated without considering the protonation constants of the Cu(II) complex. [e] pCu^{II} calculated with the protonation constants of the Cu(II) complex.

The four following protonation constants, which were accurately determined, were attributed to the first protonation of the four phosphonate functions. The second successive protonation constant for each $-PO_3H^-$ functions were assumed to be lower than 10^1 M^{-1} in agreement with numerous other systems.[21] Lastly, the $\log K^H$ value related to the pyridine unit is also estimated to be lower than 2.[19] Therefore, under our experimental conditions (starting pH ~ 2.5), ligand **L** exists as a LH_6^{2-} protonated species (Fig. 1).

The Cu(II) complexes with **L** were then characterized and quantified by pH-metric and spectrophotometric titrations *vs.* pH. Only protonated monocupric monochelates $CuLH_y$ were identified with y = 0 to 4 ($\log K_{CuLH} = 8.24(8)$, $\log K_{CuLH2} = 7.15(9)$, $\log K_{CuLH3} = 5.7(1)$, $\log K_{CuLH4} = 4.0(2)$). Interestingly, the stability constant of the Cu(II) complex with **L** is rather high and represents one of the most stable cupric species reported until now for non-macrocyclic branched compounds (to be compared for example with ethylene-diamine-tetramethylene-phosphonic acid **EDTPA** with $\log K_{CuL} = 23.2$, $pCu^{II} = 12.7$ at pH 7.4 and $[\mathbf{EDTPA}]_{tot} = 10^{-5}$ M and $[Cu(II)]_{tot} = 10^{-6}$ M).[22] This very high stability was further confirmed by determining the conditional stability constant $K^*_{CuL^*}$ under acidic conditions ($\log K^*_{CuL^*} = 4.25(4)$ and 5.82(9) at pH 1.0 and 2.0, respectively, **L*** stands for the protonated form of ligand **L** at either pH 2.0 or pH 1.0 and K^* designates the conditional stability constant). Fig. 1 displays the speciation diagrams of the protonated cupric species with **L** for a ratio $[Cu(II)]_{tot}/[L]_{tot} = 1$ as a function of pH.

If we now compare the pCu^{II} values measured at pH 7.4 (Table 1), we clearly observe that the substitution of the terminal carboxylate functions in **L′** by phosphonates in **L** induces a sizeable stabilization of the Cu(II) complex by almost two orders of magnitude, which can be related to the increase of the global amino basicity in **L** ($\log\beta_{LH2} = 21.50$) with respect to **L′** ($\log\beta_{L'H2} = 16.80$). As no protonation constants for the cupric complexes with **DO2P** are available in the reported literature, the pCu^{II} values reported in

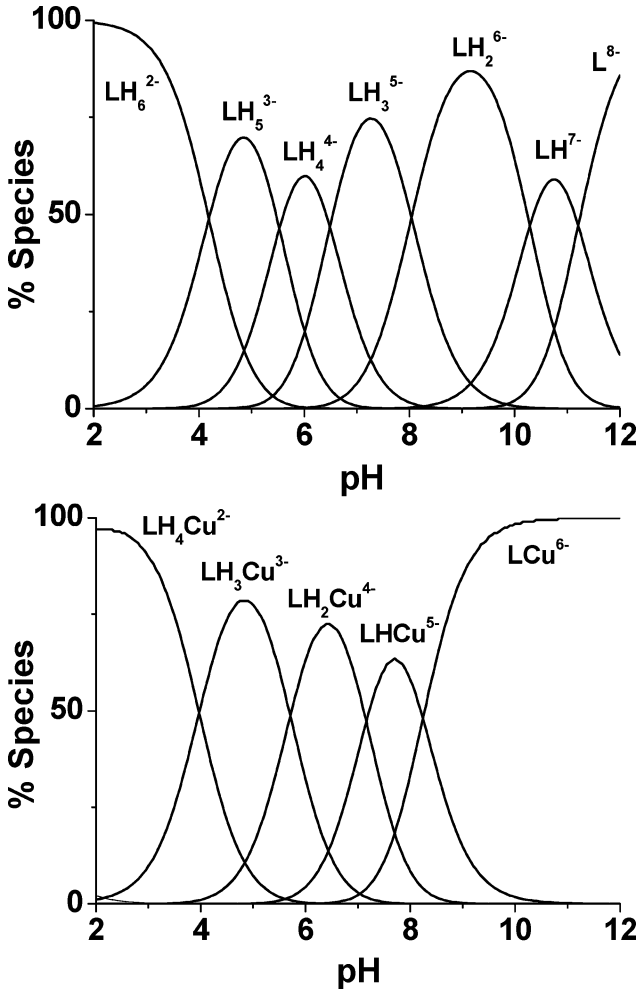


Fig. 1 Speciation diagrams of the species present in solution as a function of pH for **L** ($[\mathbf{L}]_{tot} = 2 \times 10^{-3}$ M, top) and for a stoichiometric mixture of **L** and Cu(II) ($[\mathbf{L}]_{tot} = [Cu(II)]_{tot} = 2 \times 10^{-3}$ M, bottom). Solvent: water; I = 0.1 M ($NaClO_4$), T = 25.0(2) °C.

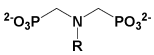
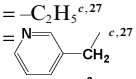
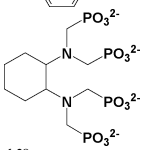
Table 1 were calculated without considering the protonated Cu(II) species for the sake of comparison. Taking into account the overall thermodynamic constants, we can calculate higher pCu^{II} values of 15.5 and 12.7 for ligand **L** and **L′**, respectively. The comparison of the pCu^{II} data measured for the tetraazacyclododecane-based ligand **DO2P** and for the non-macrocyclic compound **L** shows a stabilization of about three orders of magnitude (pCu^{II} = 17.8 and 14.4 for **DO2P** and **L**, respectively). Interestingly, this improved stability can also be related to the higher global branched amine basicity of **DO2P** ($\log\beta_{DO2PH4}$ = 23.72) by comparison with **L** ($\log\beta_{LH2}$ = 21.5) and emphasizes that, despite the lack of preorganization of **L** with respect to **DO2P**, the Cu(II) complexes with **L** are very stable species in solution. The formal stability constants of the metal complexes with M(II) = Zn(II), Ni(II) and Ga(III) have been determined by potentiometric methods and are gathered in Table 1. The corresponding stability sequence satisfactorily follows the Irving–Williams[23] order with the Cu(II) complexes being the more stable due to Jahn–Teller distortions.[24] Importantly and as anticipated,[23] the complexation of Cu(II) by **L** is very selective when compared to those of Zn(II), Ni(II) or Ga(III), with almost five orders of magnitude of difference in $\log K_{ML}$ (Table 1) in the worst case. These differences are in good agreement with those calculated for comparable polyaminomethanephosphanate derivatives (Table 3).

Table 2 Spectroscopic parameters of the protonated Cu(II) complexes formed with **L**.[a]

	Absorption bands λ_{max} (ε)/nm(M^{-1} cm^{-1})		
Species	Pyridine $\pi \rightarrow \pi^*$	N → Cu CT	Cu(II) d–d
CuL^{6-}	266 (5520)	359 (3350)	703 (236)
$CuLH^{5-}$	266 (5670)	329 (2140)	702 (153)
$CuLH_2^{4-}$	264 (5490)	315 (2395)	653 (153)

[a] Solvent: water; I = 0.1 M ($NaClO_4$); T = 25.0(2) °C.

Table 3 Formal stability constants ($\log K_{ML}$) for ligand **L**, and related polyaminomethanephosphonate analogues

L	$\log K_{CuL}$	$\log K_{NiL}$	$\log K_{ZnL}$	$\log K_{CoL}$
				
R = $-CH_3$ [a,25]	14.32	9.59 (4.73)	10.44 (3.88)	9.27 (5.05)
R = $-CH_2PO_3^{2-}$ (**NTPA**) [b,26]	17.2	11.7 (5.5)	14.6 (2.6)	14.0 (3.2)
R = $-C_2H_5$ [c,27]	13.26	8.14 (5.12)	9.33 (3.93)	7.95 (5.31)
R =  [c,27]	12.41	7.58 (4.83)	9.21 (3.2)	7.75 (4.66)
 [d,28]	9.73	3.94 (5.79)	9.12 (0.61)	3.31 (6.42)
EDTPA [b,29]	23.21	16.38 (6.83)	18.76 (4.45)	17.11 (6.1)
L [d]	22.71	16.50 (6.21)	17.84 (4.87)	nd

Solvent: H_2O; T = 25 °C. [a] I = 0.1 M (KCl). [b] I = 0.1 M KNO_3. [c] I = 0.2 M (KCl). [d] I = 0.1 M ($NaClO_4$). The values in parentheses correspond to the difference $\log K_{CuL} - \log K_{ML}$.

Fig. 2 displays the variation of the pM values (M = Cu(II), Zn(II), Ni(II) and Ga(III)) *vs.* pH, which clearly illustrates the expected

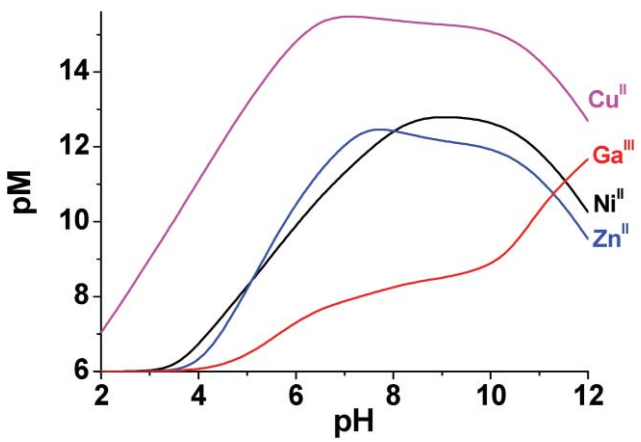


Fig. 2 Evolution of the pM values (pM = $-\log[M]_{free}$ with M = Cu(II), Zn(II), Ni(II) and Ga(III)) as a function of pH. Solvent: water; I = 0.1 M ($NaClO_4$); T = 25.0(2) °C; $[\mathbf{L}]_{tot} = 10^{-5}$ M and $[M]_{tot} = 10^{-6}$ M.

selectivity of ligand **L** toward Cu(II) with respect to the other cations studied over a wide range of acidity.

UV-Vis absorption spectroscopy

Fig. 3 displays the evolution of the UV-Vis absorption spectra of the Cu**L** complex as a function of pH, together with the corresponding electronic spectra of the Cu(II) species formed along the titration, which were calculated by statistical methods.[30] Table 2 summarizes the main spectroscopic parameters associated with these Cu(II) species. Whatever the pH, the absorption spectra are dominated by a structured band at *ca.* 265 nm ($\varepsilon_{265} \sim 5.5 \times 10^3$ M^{-1} cm^{-1}) related to the $\pi \rightarrow \pi^*$ transitions of the pyridine moiety.[31] In addition, the electronic spectra are characterized by an absorption band of weaker intensity ($\varepsilon \sim 2.0$–3.4×10^3 M^{-1} cm^{-1}), with a maximum of absorption ranging from 315 nm to 359 nm, depending on the protonation state of the Cu(II) complex. These absorption bands induced by Cu(II) complexation most likely correspond to $N_{amino} \rightarrow$ Cu(II) charge transfer (LMCT) transitions.[27a,32]

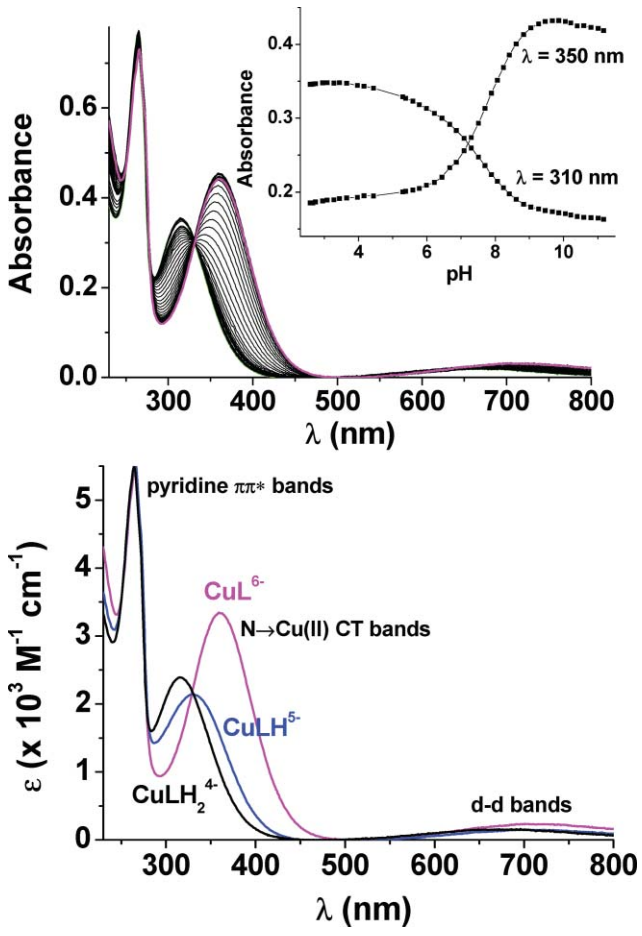


Fig. 3 Spectral changes *vs.* pH recorded for an equimolar solution of Cu(II) and **L** (water; $I = 0.1$ M ($NaClO_4$); $T = 25.0(2)$ °C; $[\mathbf{L}]_{tot} = 1.41 \times 10^{-4}$ M; $[Cu(II)]_{tot} = 1.40 \times 10^{-4}$ M, top) and electronic absorption spectra of the protonated cupric complexes with **L** (bottom). Solvent: water; $I = 0.1$ M ($NaClO_4$); $T = 25.0(2)$ °C; $[\mathbf{L}]_{tot} = 1.41 \times 10^{-4}$ M; $[Cu(II)]_{tot} = 1.40 \times 10^{-4}$ M. Inset: evolution of the absorbances at 350 and 310 nm.

The stepwise hypsochromic shifts of 30 nm and 14 nm of the LMCT band upon protonation (Fig. 3, bottom) can be ascribed to the weakening of the PO_3^{2-}–Cu(II) bonds upon protonation in favor of a strengthening of the N–Cu(II) bonds. Lastly, a broad and weak absorption band ($\varepsilon \sim 150$–240 M^{-1} cm^{-1}) was evidenced in the visible region (λ_{max} 650–700 nm) and was attributed to d–d transitions on Cu(II), indicative of an octahedral or square pyramidal geometry,[33] while d–d transitions are observed above 800 nm for triangular based bipyramidal complexes.[34] The hypsochromic shift of ~ 50 nm upon protonation of Cu**L**H^{5-} species to afford Cu**L**H_2^{4-} most likely indicates a drastic change of the Cu^{2+} coordination geometry.[33] The involvement of the N pyridine atom in the metal coordination sphere cannot be excluded and can be related to the extra-stabilization of the Cu(II) complexes with **L** ($pCu^{II} = 15.5$, Table 1) when compared with those formed with **EDTPA** ($pCu^{II} = 12.7$). Solid state structures of comparable linear or macrocyclic analogues points out the potential participation of the aromatic ring nitrogen atom to Cu(II) coordination.[19,35] Our spectroscopic data correlate with those obtained with bis- and tris-(methanephosphonate) derivatives of a 14-membered tetraaza-macrocycle containing pyridine.[19] Thorough physico–chemical studies with appropriate models are currently under progress to fully elucidate the structural and geometrical properties of these Cu(II) complexes with **L**.

Thanks to these spectroscopic probes and preliminary kinetic experiments, it was noticed that the copper complexation is instantaneous, an important criterion for the use of such compounds within the frame of ^{64}Cu and ^{67}Cu complexation for PET imaging or radiotherapeutic applications.

The 1 : 1 Cu : **L** composition was also confirmed by isolation of the Cu(II) complex and its characterization by electrospray mass spectrometry in the negative mode. The spectrum displays a major peak at $m/z = 572.955$, which unambiguously corresponds to the $[Cu\mathbf{L}H_5]^-$ species, as evidenced by its corresponding isotopic distribution. Adducts containing sodium cations were also detected (Fig. 4).

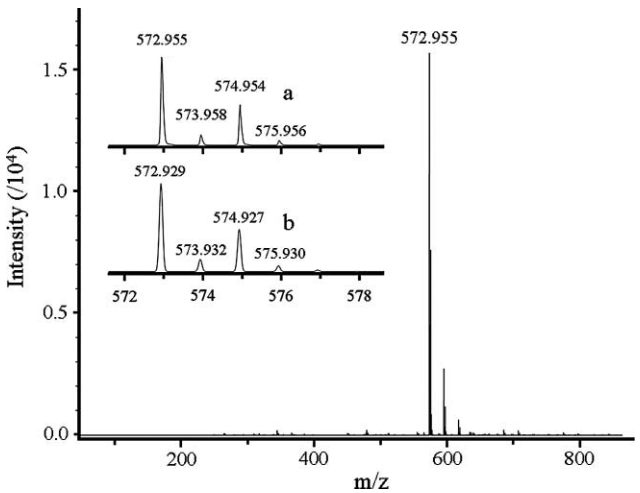


Fig. 4 ES/MS spectra of the $[Cu\mathbf{L}]Na_6$ complex in water. Inset: expansion of the region of the molecular peak (a) and its simulated pattern (b).

Discussion

In order to better understand the origin of such a strong Cu(II) chelation, we focused our attention towards the hypothesis

proposed by Lukes and co-workers, who postulated that, rather than the chemical structure and topography of the ligand, the two first pK values of the amine backbone are the dominating parameters for the complexation of Cu(II).[18] Fig. 4 summarizes a compilation of some 49 compounds available in the literature, corresponding to linear or macrocyclic branched polyaza ligands for which the logK_{CuL} and the pK are well documented. A full description of the ligands used and the corresponding references are provided in the ESI.†

As evidenced in Fig. 5, the hypothesis proposed by Lukes and co-workers[18] can be confirmed and a linear correlation of logK_{CuL} with the sum of the two first pKs can be evidenced. On the basis of a simple linear regression, we can obtain a first rough approximation of the stability constants for branched polyaza ligands with Cu(II) using the two first pK values of the ligands and the following equation :

$$\log K_{CuL} = 1.20 \times (pK_1 + pK_2) - 3.63 \ (R^2 = 0.916) \quad (1)$$

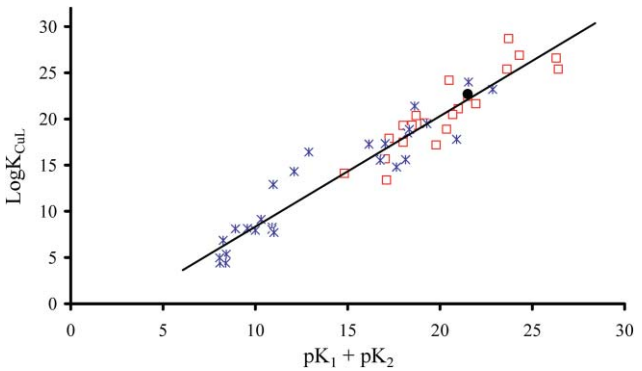


Fig. 5 Representation of the logK_{CuL} values as a function of the sum of the two first pK values of the amines of linear (stars) and cyclic (squares) branched polyaza ligands (only one pK was used for monoamines). The black dot represents the value determined for **L** by potentiometry.

Using eqn (1), the logK_{CuL} value estimated for **L** is equal to 22.2, in reasonably good agreement with the value of 22.7 determined in this study by potentiometric method.

Experimental

Synthesis of the ligand

Materials and methods

Column chromatography and flash column chromatography were performed on silica (0.063–0.200 mm, Merck) or silica gel (40–63 μm, Merck) or on standardized aluminium oxide (Merck, Activity II–III). DMF was distilled under reduced pressure. Other solvents were used as purchased. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AC 200, Avance 300 and Avance 400 spectrometers operating at 200, 300 or 400 MHz, respectively for ^{1}H. ^{31}P NMR (161.9 MHz) spectra were recorded on an Avance 400 apparatus. Chemical shifts are given in ppm, relative to residual protiated solvent.[36] IR spectra were recorded on a Nicolet 380 FT-IR spectrometer (Thermo Scientific) as solid samples. Compound **1**[14] was obtained according to literature procedures, starting from 2,6-dibromomethylpyridine, the latter being obtained according to the procedure described below.

Synthesis of 2,6-dibromomethylpyridine

2,6-Dihydroxymethylpyridine (3.70 g, 26.6 mmol) was dissolved in DMF (15 mL). The flask was immersed in an ice bath and vigorously stirred and PBr_3 (16.6 g; 5.75 mL; 61.2 mmol) was slowly added dropwise. The reaction mixture rapidly turned black. The temperature was raised to r.t. and the solution was stirred for 4 h. Water (20 mL) was slowly added and the aqueous layer was extracted with Et_2O. The organic extracts were dried over Na_2SO_4, filtered and evaporated to dryness. The solid residue was purified by column chromatography (SiO_2, CH_2Cl_2) to afford **1** as a white solid in 84% yield (5.90 g). All analyses correspond to those reported in the literature.[37]

Synthesis of compound 2

To the hydrochloride salt of 2,6-diaminomethylpyridine (810 mg, 3.3 mmol)[14] and ethanol (20 mL), was added NaOH (395 mg, 9.9 mmol). The mixture was refluxed for 1 h. The solvent was removed under reduced pressure and the resulting residue was taken up with $HPO(OEt)_2$ (1.82 g, 13.2 mmol). The mixture was stirred for 10 min in an ice bath and 1.6 mL of formaldehyde (37%) was slowly added, while keeping the temperature below 10 °C. The reaction mixture was stirred at r.t. for 30 min and at 100 °C for 14 h. The cooled solution was concentrated under reduced pressure. The crude product was purified by column chromatography over silica gel (CH_2Cl_2–MeOH gradient from 100 : 00 to 88 : 12) to give compound **2** (1.42 g, 60%) as a colourless oil. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.62 (t, J = 7.7 Hz, 1 H), 7.43 (d, J = 7.5 Hz, 2 H), 4.08 (q, J = 7.3 Hz, 16 H), 4.05 (s, 4 H), 3.17 (d, J = 10.0 Hz, 8 H), 1.27 (t, J = 7.1 Hz, 24 H). ^{13}C {^{1}H} NMR ($CDCl_3$, 50 MHz): δ 157.8, 136.9, 122.1, 62.4 (t, J = 8.0 Hz), 61.9 (d, J = 7.0 Hz), 50.2 (dd, J = 158.7 Hz, J = 8.6 Hz), 16.4 (d, J = 6.5 Hz). ^{31}P NMR ($CDCl_3$, 400 MHz): δ 24.4. IR (cm^{-1}, ATR): ν 2979 (w), 2930 (w), 2909 (w), 1680 (s), 1442 (w), 1390 (w), 1230 (s, $\nu_{P=O}$), 1160 (m), 1013 (s), 957 (s). ESI$^+$/MS (MeOH–H_2O): m/z = 738.3 ([M + H]$^+$, 100%). Anal. Calcd. for $C_{27}H_{55}N_3P_4O_{12}$, $2H_2O$: C, 41.91; H, 7.68; N, 5.43. Found: C, 41.88; H, 7.30; N, 5.80.

Synthesis of LH_8

Compound **2** (650 mg, 0.88 mmol) was dissolved in dichloromethane (10 mL) and TMSBr (4.7 mL, 35.2 mmol) was added. The solution was stirred at r.t. for 24 h. A second addition of TMSBr (4.7 mL, 35.2 mmol) was made and the mixture was stirred at r.t. for 24 h. The solvent was removed under reduced pressure, the resulting residue was taken up with MeOH (10 mL) and stirred for 2 h at r.t. The evaporation, dissolution procedure was repeated with MeOH (10 mL) and the solution was stirred for 24 h at r.t. The solvent was removed under vacuum and the residue was dissolved in a minimum of MeOH. Addition of Et_2O resulted in the formation of a precipitate, which was collected by centrifugation and dried under vacuum to afford ligand $H_8\mathbf{L_1}$ with 81% yield (450 mg). ^{1}H NMR (D_2O, 300 MHz): δ 7.96 (t, J = 7.8 Hz, 1 H), 7.50 (d, J = 7.8 Hz, 2 H), 4.95 (s, 4 H), 3.64 (d, J = 12.4 Hz, 8 H). ^{13}C {^{1}H} NMR (D_2O, 75 MHz): δ 149.4, 139.9, 123.9, 59.3, 52.3 (d, J = 137.8 Hz). ^{31}P NMR (D_2O, 400 MHz): δ 7.98. IR (cm^{-1},

ATR): *ν* 2940 to 2580 (w, br, ν_{OH}), 1618 (m), 1426 (m), 1160 (s, ν_{P-O}), 919 (s, $\nu_{P=O}$). Anal. Calcd. for $C_{11}H_{23}N_3O_{12}P_4.HBr{\cdot}2H_2O$: C, 20.96; H, 4.48; N, 6.66. Found: C, 21.06; H, 4.48; N, 6.11.

Synthesis and characterization of $[CuL]Na_6{\cdot}7H_2O$

To a solution of ligand **L** (47.7 mg, 0.09 mmol) in H_2O (2 mL) was added $CuCl_2{\cdot}2H_2O$ (15.8 mg, 0.09 mmol) dissolved in H_2O (2 mL), resulting in a deep blue solution. The mixture was stirred for 1 h at r.t. The pH was raised with a diluted aqueous NaOH solution and the solution was concentrated to *ca.* 0.5 mL. Addition of acetone resulted in the formation of a greenish precipitate, which was collected by centrifugation and dried under reduced pressure to yield $[CuL]Na_6{\cdot}7H_2O$ (52 mg, 91%). Anal. Calcd. for $C_{11}H_{15}CuN_3Na_6O_{12}P_4{\cdot}7H_2O$: C, 15.86; H, 3.51; N, 5.05. Found: C, 15.88; H, 3.48; N, 4.94. ESI$^-$/MS (MeOH–H_2O): m/z = 572.955 ($[CuLH_5]^-$, 100%); 595.7 ($[CuLH_4Na]^-$, 15%). IR (cm^{-1}, ATR): *ν* 3185 (m, br, ν_{OH}), 1644 (m), 1604 (m) 1442 (m), 1117 (s, ν_{P-O}), 1037 (s, ν_{P-O}), 963 (s, $\nu_{P=O}$).

Physico–chemical studies

Starting materials and solvents

Copper(II) perchlorate ($Cu(ClO_4)_2{\cdot}6H_2O$, Fluka, purum p.a.), zinc(II) perchlorate ($Zn(ClO_4)_2{\cdot}6H_2O$, Ventron, Alfa Produkte, 98.9%), nickel(II) perchlorate ($Ni(ClO_4)_2{\cdot}6H_2O$, Fluka, purum p.a.) and gallium(III) nitrate ($Ga(NO_3)_3.xH_2O$, Alfa Aesar, Puratronic®) are commercial products, which were used without further purification. Distilled water was further purified by passing it through a mixed bed of ion-exchanger (Bioblock Scientific R3-83002, M3-83006) and activated carbon (Bioblock Scientific ORC-83005) and was de-oxygenated by CO_2- and O_2-free argon (Sigma Oxiclear cartridge) before use. All the stock solutions were prepared by weighing solid products using an AG 245 Mettler Toledo analytical balance (precision 0.01 mg). The ionic strength was maintained at 0.1 M with sodium perchlorate ($NaClO_4{\cdot}H_2O$, Merck, p.a.), and all the measurements were carried out at 25.0(2) °C. The metal contents of the solutions were ascertained according to classical colorimetric titrations.[38]

CAUTION! Perchlorate salts combined with organic ligands are potentially explosive and should be handled in small quantities and with the adequate precautions.[39]

Potentiometric titrations

The potentiometric titrations of ligand **L** ($2.2–2.5 \times 10^{-3}$ M) and its metal complexes ($[M]_{tot}/[L]_{tot} \sim 1$) were performed using an automatic titrator system 794 Basic Titrino (Metrohm) with a combined glass electrode (Metrohm 6.0234.500, Long Life) filled with 0.1 M NaCl in water and connected to a microcomputer (Tiamo light 1.2 program for the acquisition of the potentiometric data). The combined glass electrode was calibrated as a hydrogen concentration probe by titrating known amounts of perchloric acid ($\sim 1.3 \times 10^{-2}$ M from $HClO_4$, Fluka, ~ 70%) with CO_2-free sodium hydroxide solution ($\sim 10^{-1}$ M from NaOH, BdH, AnalaR).[40] The $HClO_4$ and NaOH solutions were freshly prepared just before use and titrated with sodium tetraborate decahydrate ($B_4Na_2O_7{\cdot}10H_2O$, Fluka, puriss, p.a.) and potassium hydrogen phthalate ($C_8H_5KO_3$, Fluka, puriss, p.a.), respectively, using methyl orange and with phenolphthalein (Prolabo, purum) as the indicators. The cell was thermostated at 25.0(2) °C by the flow of a Lauda E200 thermostat. A stream of Argon, pre-saturated with water vapor, was passed over the surface of the solution. The Glee program[40] was applied for the glass electrode calibration (standard electrode potential E_0/mV and slope of the electrode/mV pH^{-1}) and to check carbonate levels of the NaOH solutions used (< 5%). The potentiometric data of **L** and its metal complexes (about 300 points collected over the pH range 2.5–11.5) were refined with the Hyperquad 2000[41] program which uses non-linear least-squares methods.[42] Potentiometric data points were weighted by a formula allowing greater pH errors in the region of an end-point than elsewhere. The weighting factor W_i is defined as the reciprocal of the estimated variance of measurements: $W_i = 1/\sigma_i^2 = 1/[\sigma_E^2 + (\delta E/\delta V)^2\sigma_V^2]$ where σ_E^2 (0.1 mV) and σ_V^2 (0.005 mL) are the estimated variance of the potential and volume readings, respectively. The constants were refined by minimizing the error-square sum, U, of the potentials:

$$U = \sum_i^N W_i(E_{obs,i} - E_{cal,i})^2$$

At least three titrations were treated either as single sets or as separated entities, for each system, without significant variation in the values of the determined constants. The quality of fit was judged by the values of the sample standard deviation, S, and the goodness of fit, χ^2 (Pearson's test). At σ_E = 0.1 mV (0.023 σ_{pH}) and σ_V = 0.005 mL, the values of S in different sets of titrations were between 0.8 and 1.2, and χ^2 was below 22. The scatter of residuals *vs.* pH was reasonably random, without any significant systematic trends, thus indicating a good fit of the experimental data. The successive protonation constants were calculated from the cumulative constants determined with the program. The uncertainties in the log$K$ values correspond to the added standard deviations in the cumulative constants. The distribution curves of the protonated species of **L** and its metal complexes as a function of pH were calculated using the Hyss2009 program.[43]

Spectrophotometric titrations *vs.* pH

Spectrophotometric titration as a function of pH of the free ligand **L** was first performed. Stock solution of **L** (1.51×10^{-4} M) was prepared by quantitative dissolution of a solid sample in deionised water and the ionic strength was adjusted to 0.1 M with $NaClO_4$ (Fluka, puriss). An aliquot of 40 mL of the solution was introduced into a jacketed cell (Metrohm) maintained at 25.0(2) °C (Lauda E200 thermostat). The free hydrogen ion concentration was measured with a combined glass electrode (Metrohm 6.0234.500, Long Life) and an automatic titrator system 794 Basic Titrino (Metrohm). The Ag/AgCl reference glass electrode was filled with NaCl (0.1 M, Fluka, p.a.) and was calibrated as a hydrogen concentration probe as described above. The initial pH was adjusted to ~ 2 with $HClO_4$ (Fluka, ~ 70%), and the titration of the free ligand **L** (2.5 < pH < 11.2) was then carried out by addition of known volumes of NaOH solutions (BdH, AnalaR) with an Eppendorf microburette. Special care was taken to ensure that complete equilibration was attained. Absorption spectra *vs.* pH were recorded using a Varian Cary

50 spectrophotometer fitted with Hellma optical fibers (Hellma, 041.002-UV) and an immersion probe made of quartz suprazil (Hellma, 661.500-QX). Spectrophotometric titration of the cupric complexes with **L** was thereafter carried out. About one equivalent of Cu(II) perchlorate ($[Cu(II)]_{tot}$ = 1.40 × 10^{-4} M) was added to 40 mL of **L** (1.41 × 10^{-4} M) in a jacketed cell (Metrohm) maintained at 25.0(2) °C. The initial pH was adjusted to ~ 2–3 with $HClO_4$ (Fluka, ~70%), and the titration of the cupric complexes (2.59 < pH < 11.17) was then carried out by addition of known volumes of NaOH solutions (BdH, AnalaR) with an Eppendorf microburette. Special care was also taken to ensure that complete equilibration was attained. Absorption spectra *vs.* pH were recorded using the Varian Cary 50 spectrophotometer described above.

Spectrophotometric titrations of L by Cu(II) at fixed pH

Stock solutions of **L** (~ 2.5 × 10^{-3} M) were prepared in water and then freshly diluted with $HClO_4$ (10^{-2} M or 10^{-1} M) to obtain a ligand concentration of ~ 2 × 10^{-4} M. The ionic strength was kept constant at 0.1 M with either sodium perchlorate ($NaClO_4 \cdot H_2O$, Merck, p.a.) or perchloric acid (Fluka, ~70%). The spectrophotometric titrations of **L** by Cu(II) were thus carried out on solutions at pH ~ 2 (10^{-2} M $HClO_4$) and at pH ~ 1.0 (10^{-1} M $HClO_4$). Microvolumes of a concentrated solution of Cu(II) (4.4 × 10^{-3} M) were added to 2 mL of the ligand solutions in a 1 cm path length optical cell (the $[Cu]_{tot}/[L]_{tot}$ ratio varied from 0 to 2). Special care was taken to ensure that complete equilibration was attained. The corresponding UV-Vis spectra were recorded from 230 nm to 800 nm on a Cary 300 (Varian) spectrophotometer maintained at 25.0(2) °C by the flow of a Lauda E200 thermostat.

Analysis and processing of the spectroscopic data

The spectrophotometric data were analyzed with Specfit[30a–c] program which adjusts the absorptivities and the stability constants of the species formed at equilibrium. Specfit uses factor analysis to reduce the absorbance matrix and to extract the eigenvalues prior to the multi-wavelength fit of the reduced data set according to the Marquardt algorithm.[30d,e]

Conclusions

In conclusion, we have developed a straightforward synthesis of a new non-macrocyclic ligand which forms very stable complexes with Cu(II). The kinetics of Cu(II) complexation is fast and the Cu(II) coordination is very selective for Cu(II) with respect to Ni(II) and Zn(II), its neighbours in the *d* series. These coordination properties thus represent a very promising approach for copper(II) complexation with high potential within the frame of ^{64}Cu chelation for PET imaging and radiotherapy. Current efforts are now directed toward a better understanding of the coordination mode and the introduction of a labeling function for grafting on biological targets.

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