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Non-symmetrical bis(aminoalkyl)phosphinates: new ligands with the enhanced binding of Cu(II) ions

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Abstract

Three novel, non-symmetrical bis(aminoalkyl)phosphinic acids, L^1-L^3, have been synthesized and characterized. Solution studies on the coordination abilities of the ligands have shown that these compounds form variously protonated mono- and bis-complexes, where copper (II) coordination is realized through the nitrogen atom(s) of the amino group(s), supported by oxygen(s) from the phosphinate unit(s). Potentiometric titrations and a full spectroscopic analysis clarified the species distribution profiles and detailed coordination modes. The results show that L^1-L^3 ligands are efficient chelating agents for Cu(II) ions; their metal binding abilities were compared to structurally related compounds described earlier in the literature.

Keywords: Ligand design, aminophosphinic acids, Cu(II) complexes, stability constants

1. Introduction

Aminophosphinic acids, phosphorus analogues of natural amino acids and short peptides comprising C-P(O)(OH)-C structural motif, have attracted significant consideration because of their notable biological activity. Accordingly, aminophosphinic acids have been studied as potent antimicrobial, antiviral, antitumor, and neuromodulatory agents.[1] These activities basically originate from inhibition of enzymes of different class, in particular, proteases and ligases.[2, 3] The most recognized, recent achievements in enzyme inhibition involved phosphinic peptide analogs and metallo-containing proteases of biomedical importance, e.g. representatives of matrix metalloproteinase family, and mammalian, bacterial and protozoal aminopeptidases.[4, 5]

The presence of an additional α-amino group in the fundamental aminophosphinic motif results with an enhanced potential of structural diversification of biologically active compounds. Bis(aminomethyl)phosphinic acids that can be symmetrically or non-symmetrically substituted at the α-carbon and/or nitrogen atom(s) offer an opportunity to refine steric complementarity of competitive ligands with the active site of enzymatic targets, and binding affinity to catalytic metal ions of metallo-dependent proteins. Following this concept, non-symmetrical and C_2-symmetrical phosphinic peptides were synthesized and found very potent inhibitors of HIV1 protease.[6-8] The N-C-P-C-N backbone is also present in the structures of high-affinity ligands of the M1 alanyl aminopeptidase (Scheme 1), a zinc-dependent exopeptidase involved in angiogenesis and tumor invasion.[9, 10] Recently, we described *N*-alkyl-bis(aminomethyl)phosphinic inhibitors of urease (Scheme 1), a hydrolase responsible for global nitrogen circulation by urea cleavage, which among others is a virulence factor of digestive and urinary tract pathogens.[11, 12]

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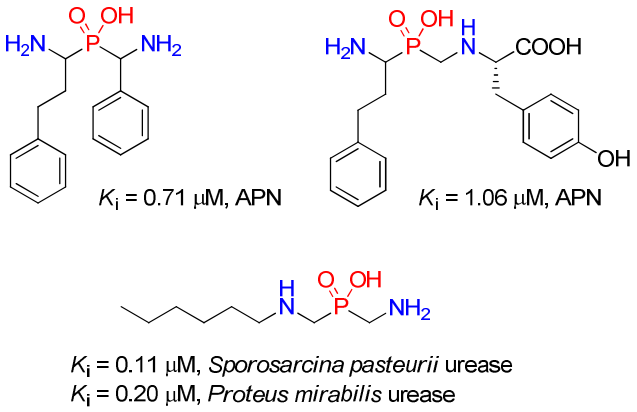
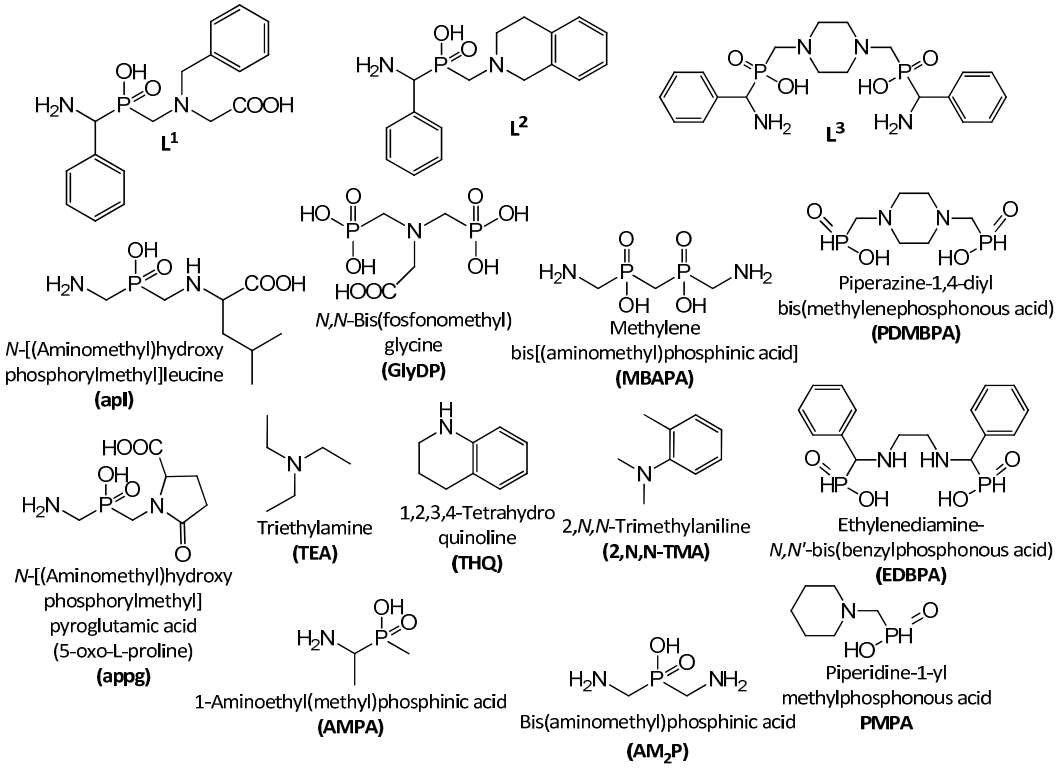


Scheme 1. Exemplified structures of competitive inhibitors which comprise bis(aminoalkyl)phosphinic acid motif and are targeted towards metal-containing hydrolases: alanyl aminopeptidase and bacterial ureases.

Apart from biological activity, structurally diverse aminophosphinic acids have been considered as ligands for metal ion complexation.[13-15] In the particular case of the N-C-P-C-N scaffold, the most systematic studies involved cyclic and acyclic bis(aminometyl)phosphinates lacking substituents of the α-carbon atoms.[16-18] These polydentate ligands, especially symmetrical phosphinate-containing heterocycles functionalized additionally with carboxylate groups, the derivatives of glycine or iminodiacetic acid, were studied with a range of trivalent lanthanide and biologically relevant divalent metal ions.[18, 19] The non-symmetrical and α-substituted N-C-P-C-N derivatives have been characterized to much less extent.[20, 21] As an extension to those previous complexation studies performed in our laboratories, we present here the coordination ability of three novel bis(aminoalkyl)phosphinic acid derivatives containing a tertiary amino group (N,N-dialkyl ligand L^1 and azacyclic compounds L^2 and L^3, Scheme 2) towards Cu(II) ions.



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Scheme 2. Structures of ligands studied and discussed.

2. Experimental

2.1. Materials

All chemicals were of analytical grade and were used without further purification. Depending on the solubility of studied ligands, the solution studies were performed in water (L^1 and L^3) or in a MeOH/H_2O (80:20 w/w) mixture (L^2). Measurements requiring ionic strength were performed in 0.1 M $NaClO_4$. The solution of copper (II) ions were prepared by dissolving $Cu(ClO_4)_2 \cdot 6H_2O$ in water and standardized by ICP-AES. $HClO_4$ solution was titrated by standardized NaOH in MeOH/H_2O solution. Carbonate-free NaOH solution was standardized by titration with potassium hydrogen phthalate. *Caution! Perchlorate salts combined with organic ligands are potentially explosive and should be handled in small quantity and with the necessary precautions.*

2.2. Ligand synthesis

2.2.1. General synthetic procedure.[10] α-(*N*-Benzyloxycarbonylamino)benzyl-*H*-phosphonous acid, 0.92 g, 3.0 mmol)[22] and a secondary amine or amino acid (4.5 mmol, 1.5 eq.; for piperazine: 1.5 mmol, 0.5 eq., 0.13 g) were dissolved in a hot water/acetic acid/HCl_{concd} mixture (10/10/0.5 mL). Formaldehyde (36-38% aqueous solution, 6.0 mmol, 2.0 eq., 0.55 mL) was added to the clear solution. Following addition the mixture was stirred for 1 h and refluxed for 3 h, then cooled to room temperature and left for crystallization overnight at 4°C. The precipitated white solid was filtered, wash with water and dried in the air. The Cbz protection was removed by the action of HBr (33% solution in AcOH, 10 mL per 1 g) for 2 h at room temperature. The volatile acids were removed under reduced pressure. The residue was triturated with diethyl ether, the white solid was filtered, washed with ether and dried in the air.

The ^1H and ^{13}C NMR spectroscopic experiments were performed on a Jeol JNM-ECZ 400S Research FT NMR Spectrometer (JEOL Ltd., Tokyo, Japan) operating at 399.78 MHz (^1H) MHz and 100.52 MHz (^{13}C). The ^{31}P experiments were performed on a Bruker Ultrashield Spectrometer (Bruker, Rheinstetten, Germany) operating at 121.5 MHz ($^{31}P\{^1H\}$). Measurements were made in D_2O solutions supplied by ARMAR AG (Dottingen, Switzerland). Chemical shifts are reported in ppm relative to TMS and 85% H_3PO_4, used as external standards. Mass spectra were recorded using a Waters LCT Premier XE mass spectrometer (method of ionization ESI).

2.2.2. L^1: *N*-{[α-Aminobenzyl(hydroxy)phosphoryl]methyl}-*N*-benzylaminoacetic acid. Yield 48%. ^1H NMR (ppm, D_2O, 399.78 MHz) δ: 7.26-7.39 (m, 10H, 2 × C_6H_5), 4.38 (d, *J* = 11.6 Hz, 1H, CH), 4.27 (s, 2H, CH_2), 3.71 (s, 2H, CH_2), 3.18 (d, *J* = 8.4 Hz, 2H, CH_2). ^{13}C NMR (ppm, D_2O, 100.52 MHz) δ: 169.17, 131.35, 131.03 (d, *J* = 4.0 Hz), 130.52, 129.52, 129.50, 129.42, 128.37, 127.80 (d, *J* = 5.0 Hz), 60.64 (d, *J* = 3.0 Hz), 55.80 (d, *J* = 4.0 Hz), 54.88 (d, *J* = 97.5 Hz), 50.18 (d, *J* = 92.5 Hz). ^{31}P NMR (ppm, D_2O, 121.5 MHz) δ: 19.33. HRMS (ESI) m/z calcd for $C_{17}H_{22}N_2O_4P$ $[M+H]^+$: 349.1312, found: 349.1346.

2.2.3. L^2: α-Aminobenzyl[(tetrahydroisoquinoline-1-yl)methyl]phosphinic acid. Yield 70%. ^1H NMR (ppm, D_2O, 399.78 MHz) δ: 7.37 (m, 5H, C_6H_5), 7.13-7.18 (m, 3H, 3 × CH_{ar}), 6.97 (m, 1H, CH_{ar}), 4.48 (d, *J* = 11.2 Hz, 1H, CH), 4.24 (AB system, *J* = 14.0 Hz, 2H, CH_2), 3.61 (m, 1H, CH), 3.28 (m, 3H, CH + CH_2), 3.05 (m, 1H, CH), 2.91 (m, 1H, CH). ^{13}C NMR (ppm, D_2O, 100.52 MHz) δ: 131.11 (d, *J* = 4.0 Hz), 130.27, 129.61, 129.58, 128.74, 128.48, 127.96 (d, *J* = 5.0 Hz), 127.13, 126.78, 126.77, 55.39 (d, *J* = 24.1 Hz), 54.92 (d, *J* = 78.5 Hz), 52.32, 52.28, 24.52. ^{31}P NMR (ppm, D_2O, 121.5 MHz) δ: 19.07. HRMS (ESI) m/z calcd for $C_{17}H_{22}N_2O_2P$ $[M+H]^+$: 317.1413, found: 317.1403.

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2.2.4. L^3: (Piperazine-1,4-diyl)bis[α-aminobenzyl(methyl)phosphinic acid]. Yield 71%. ^{1}H NMR (ppm, D_2O + NaOD, 399.78 MHz) δ: 7.28 (m, 8H, 8 × CH_{ar}), 7.21 (m, 2H, 2 × CH_{ar}), 4.60 (d, *J* = 12.0 Hz, 2H, 2 × CH), 2.23-2.78 (m, 12H, 6 × CH_2). ^{13}C NMR (ppm, D_2O+ NaOD, 100.52 MHz) δ: 138.51, 128.39 (d, *J* = 2.0 Hz), 127.50 (d, *J* = 4.0 Hz), 127.06 (d, *J* = 2.0 Hz), 56.70 (d, *J* = 93.5 Hz), 55.38 (d, *J* = 105.6 Hz), 54.19. ^{31}P NMR (ppm, D_2O+ NaOD, 121.5 MHz) δ: 35.13. HRMS (ESI) m/z calcd for $C_{20}H_{31}N_4O_4P$ $[M+H]^+$: 453.1815, found: 453.1711.

2.3. Physical measurements

2.3.1. Potentiometric titrations of ligands and their complexes were performed using an automatic titrator system Titrando 905 (Metrohm) with a combined glass electrode (Mettler Toledo InLab Semi-Micro) calibrated daily in hydrogen ion concentration using $HClO_4$. The electrode of the organic solvent system was filled with 0.1 M NaCl in MeOH/H_2O (80:20 w/w) and conditioned two weeks before the first measurements were made. Between measurements electrode was stored in the same electrolyte solution. The pK_w in the MeOH/H_2O 80:20 w/w system was -14.42.[23] The experiments were carried out under argon atmosphere at 25±0.2˚C. All potentiometric titrations were performed on 3 ml samples with the ligand concentration of $2\cdot10^{-3}$ M and 1:1 and 1:2 Cu(II) to ligand molar ratios in pH range 2-11. For L^3 also the 2:1 Cu(II) to ligand ratio was performed. For L^1 no precipitation occurred in the entire pH range. For L^2 in 1:1 and for L^3 in 2:1 Cu(II) to ligand molar ratio a bluish precipitate occurred above pH 7 and pH 6, respectively, and these pH ranges were not included in the data processing. The exact concentration of the ligand was determined using the method of Gran.[24] The potentiometric data were refined with Superquad program.[25] The overall protonation constants β_n are concentration constants and are defined by $\beta_n = [H_nL] / ([H]_n \times [L])$ (stepwise protonation constants are defined as $\log K_1 = \log\beta_1$; $\log K_n = \log\beta_n - \log\beta_{n-1}$). The overall stability constant are defined by $\beta_{hlm} = [M_mH_hL_l] / ([M]_m \times [H]_h \times [L]_l)$. In the calculations of complex stability constants the protonation constants of free ligands (Table 2), and the hydrolysis constants related to $Cu(OH)^+$ ($\log \beta_{11} = -8.1$), $Cu(OH)_2$ ($\log \beta_{12} = -16.1$), $Cu(OH)_3^-$ ($\log \beta_{13} = -26.7$) and $Cu(OH)_4^{2-}$ ($\log \beta_{14} = -39.6$) species were taken into account.[26]

2.3.2. UV-Vis spectroscopy. All absorption spectra were recorded on Varian CARY 300 UV-Vis spectrophotometer at 25.0±0.2˚C. pH-dependent UV-Vis titrations were performed in the pH range 2-11. The combined glass electrode (Mettler Toledo InLab Semi-Micro) was prepared as in the case of potentiometric titration calibrated with buffers prepared in MeOH/H_2O (80:20 w/w) mixture before every measurement.[27, 28] The concentration of the ligand was around $2\cdot10^{-3}$M. The starting pH was adjusted to around 2 with $HClO_4$ and the titration was carried out in a cell with 1 cm optical path length. For Cu(II) complexes, titrations were performed in a 1 cm cell with 3.2 ml as total volume of the solution containing 1:1 and 1:2 molar ratios of Cu(II):ligand for L^1 and L^2, and additionally 2:1 for L^3.

2.3.3. EPR spectroscopy. EPR spectra were recorded on a Bruker ELEXSYS E500 CW-EPR spectrometer supplied with frequency counter (E 41 FC) at X-band frequency and an NMR teslameter (ER 036TM), at 77K. The solutions for EPR were prepared by using MeOH/H_20 mixture (80/20 w/w) for L^2 ligand and H_2O/ethylene glycol mixture (3:1 v/v) for L^1 and L^3. pH-dependent EPR titrations were performed in the pH range 2-11, for 1:1 and 1:2 molar ratios of Cu(II):ligand for L^1 and L^2, and additionally 2:1 for L^3. The parameters of experimental spectra were calculated through spectra simulation in Doubletnew (EPR OF S=1/2) program by Dr. Andrew Ozarowski, National High Field Magnetic Laboratory, University of Florida.

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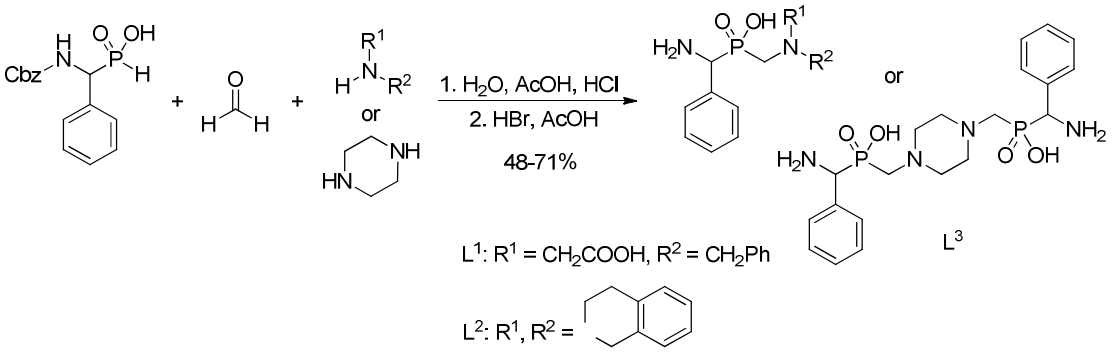
2.3.4. Mass spectrometry. Electrospray ionization mass spectrometry (ESI-MS) data were collected using a BrukerMicrO-TOF-Q spectrometer and a Bruker apex ultra FT-ICR mass spectrometer (BrukerDaltonik, Germany), equipped with an Apollo II electrospray ionization source with an ion funnel. Stock solutions were prepared by using MeOH/H_2O mixture (80/20 w/w) as a solvent. The Cu(II) to ligand molar ratio was 1:2 and the pH of the solutions was 8. The instrument parameters were: dry gas–nitrogen, temperature 200°C, ion source voltage 4500 V, collision energy 10 eV. The calibration of the instrument was performed using the Tunemix mixture (BrukerDaltonik, Germany) in the quadratic regression mode. The spectra were recorded both in the positive and negative ion mode in the range 100 to 1500 m/z and analyzed with Bruker Compass DataAnalysis 4.0 software. The overall charge of the analyzed ion was calculated on the base of the distance between the isotopic peaks.

3. Results and discussion

3.1. Design and synthesis

To obtain non-symmetrically substituted bis(aminoalkyl)phosphinic acid ligands, a three-component Mannich-type condensation[29] (the Kabachnik-Fields reaction[30]) was employed with N-protected benzylphosphonous acid as the key substrate. The structure of the added aminomethyl portion was determined by a secondary amine/amino acid used. The reaction of these starting materials with formaldehyde was performed in acidic conditions and gave solid products in a moderate to good yield (48-71%, Scheme 3). Deprotection of the N-termini (Cbz removal) proceeded quantitatively under the action of hydrobromic acid.



Scheme 3. Phosphinomethylation of a secondary amino grup with *H*-phosphinic acid and formaldehyde, followed by the N-termini deprotection.

3.2. Speciation studies

The acid-base properties of studied ligands were determined in order to evaluate their coordination properties toward copper (II) ions.

3.2.1. Ligands' protonation constants. Protonation macroconstants of studied ligands were determined by potentiometric titrations in pH range of 2-11, and obtained values are presented in Table 1. The deprotonated form of the ligand L^1, $[L]^{2-}$, may attach four protons, to two amine groups, and the carboxylate and phosphinate residues. However, in the measured pH range, three protonations steps were observed, with logK_1 = 11.11, logK_2 = 9.06 and logK_3 = 5.02. The protonation constant of the phosphinic PO(OH) group is too low (< 2) to be determined by potentiometric

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measurements. As the protonation processes of the two amine groups overlap, unequivocal assignment of the protonation macroconstants to either of the groups is impossible without determination of microconstants by NMR titrations. However, the value of $\log K_1$ = 11.11 is similar to that reported to TEA (10.78)[31] and GlyDP ligand (11.3),[15] suggesting that this log*K* corresponds to tertiary amine group. The values $\log K_2$ = 9.06 and $\log K_3$ = 5.02 may be assigned to primary amine and carboxylate groups, respectively, comparing to apl ligand with log*K* values 8.82 and 1.98 (Table 1, Table S1).[20] The less acidic character of carboxylate residue in L^1, can be explained by a smaller withdrawing effect of the tertiary amine group, in comparison to the one caused by secondary amine in apl.

The deprotonated form of L^2, $[L]^-$, may also attach three protons in the measured pH range (Table 1). The $\log K_1$ = 8.38 and $\log K_3$ = 2.12 are related to proton dissociation from the primary amine and phosphinate group, respectively. The calculated values are higher than those reported to other aromatic aminophosphinates.[13] The increase of protonation constants for amine and phosphinic group results from the use of the MeOH/H_2O (80/20 w/w) mixed solvent.[32] The change of solvent was necessary due to solubility problems of L^2 Cu(II) complexes (vide infra). The second protonation constant ($\log K_2$ = 5.46) corresponds to tetrahydroisoquinoline N atom and is similar to that reported to THQ and 2,N,N-TMA (Table 1, Table S1).[33, 34]

Table 1. Protonation constants ($\log K^H$) of L^1 – L^3 ligands,[a] and selected analogous compounds.

Species	Logβ	$\log K^H$	Protonation site	Reference
L^1				This work
LH^-	11.11(1)		$N_{Tertiary\ amine}$	
LH_2	20.17(1)	9.06	$N_{Primary\ amine}$	
LH_3^+	25.19(1)	5.02	$O_{Carboxylate}$	
L^2				This work
LH	8.38(1)		$N_{Primary\ amine}$	
LH_2^+	13.84(6)	5.46	$N_{Isoquinoline}$	
LH_3^{2+}	15.96(2)	2.12	$O_{Phosphinate}$	
L^3				This work
LH^-	8.75(1)		$N_{Primary\ amine}$	
LH_2	16.50(1)	7.75	$N_{Primary\ amine}$	
LH_3^+	22.36(2)	5.86	$N_{Piperazine}$	
LH_4^{2+}	25.29(2)	2.93	$N_{Piperazine}$	
Apl				20
LH	8.82		$N_{Primary\ amine}$	
LH_2	15.37	6.55	$N_{Secondary\ amine}$	
LH_3	17.35	1.98	$O_{Carboxylate}$	
Appg				20
LH	8.19		$N_{Primary\ amine}$	
LH_2	10.98	2.79	$O_{Carboxylate}$	
MBAPA				35
LH	9.49		$N_{Primary\ amine}$	
LH_2	18.29	8.80	$N_{Primary\ amine}$	
LH_3	20.38	2.09	$O_{Phosphinate}$	
AMPA				13

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LH	8.29		$N_{Primary\ amine}$	
LH_2	9.29	1.0	$O_{Phosphinate}$	16
AM_2P				
LH	8.509		$N_{Primary\ amine}$	
LH_2	15.580	7.071	$N_{Primary\ amine}$	
LH_3	16.35	0.77	$O_{Phosphinate}$	36
EDBPA				
LH	7.58		$N_{Secondary\ amine}$	
LH_2	11.90	4.32	$N_{Secondary\ amine}$	37
PDMBPA				
LH	6.719		$N_{Piperazine}$	
LH_2	9.341	2.622	$N_{Piperazine}$	37
PMPA				
LH	8.41		$N_{Piperidine}$	15
GlyDP				
LH	11.3		$N_{Tertiary\ amine}$	31
TEA				
LH	10.78		$N_{Tertiary\ amine}$	33, 34
2,N,N-TMA				
LH	5.86		$N_{Tertiary\ amine}$	33
THQ				
LH	5.03		$N_{Secondary\ amine}$	

[a] I = 0.1 M ($NaClO_4$), T = (25.0 ± 0.2) °C, solvent: H_2O for L^1 and L^3, and MeOH/H_2O 80:20 w/w for L^2.

The L^3 ligand behaves in measured pH range like a four-protonic acid, with protonation constants logK_1 = 8.75 and logK_2 = 7.75 most probably corresponding to primary amine groups, and logK_3 = 5.86 and logK_4 = 2.93 related to deprotonation of piperazine N atoms (Table 1, Table S1). The obtained values are in a good agreement with pK values reported earlier for PDMBPA,[37] MBAPA,[35] and aromatic aminophosphinates.[13] The protonations of both phosphinic PO(OH) groups occur much below pH 2 and constants corresponding to these processes were not determined under our experimental conditions.

3.2.2. Stoichiometry of Cu(II) complexes. Formation of complexes in solution upon mixing L^1, L^2 and L^3 ligands with Cu(II) ions was in the first place monitored using ESI-MS spectrometry. Although ESI-MS is not able to distinguish the ionizable protons in the species, this method can be successfully applied to determine the metal-to-ligand stoichiometry directly from the m/z values, and has been employed by us for this purpose on previous occasions.[38-41] Analysis of the ESI-MS spectra of the reaction mixture of Cu(II):L^1 showed the formation of monomeric species, successfully attributed to $\{[CuL^1] + ClO_4^-\}^-$ (m/z 507.9815). Similar behaviour has been observed for Cu(II):L^3 system, where appearing species was interpreted as $\{[CuL^3H]^+\}^+$ (m/z 514.0933). The presence of CuL_2 species was not observed. The ESI-MS spectra of the reaction mixture of Cu(II):L^2 indicated the formation of the following complexes: $\{[CuL^2_2] + Na^+\}^+$ (m/z 716.1787), $\{[CuL^2_2] + K^+\}^+$ (m/z 732.1533), $\{[Cu_2L^2_4] + H^+\}^+$ (m/z 1389.3961) and $\{[Cu_2L^2_4] + Na^+\}^+$ (m/z 1411.3896).

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3.2.3. Stability constants of Cu(II) complexes. To determine the complex formation properties of L^1, L^2 and L^3 towards Cu(II) ions, an informative combination of potentiometry, UV-Visible and Electron Paramagnetic Resonance spectroscopies was used. Potentiometric titrations gave insight into the thermodynamic parameters of the studied complexes, allowing us to compare their stability and pH-dependent distribution to other, relevant phoshinic ligands. UV-Vis and EPR were helpful to precisely identify the binding sites, the donor atoms and the coordination geometry of complex species formed in solution. The EPR spectra recorded in magnetic field range 0 - 5000 G for Cu(II) complexes with L^1-L^3 ligands did not give any indication for the formation of dimeric forms (the characteristic hyperfine splitting should be seen for the two different resonance transitions: the "forbidden" at ~1500 G and the "allowed" ~2400 G, due to the interaction of the unpaired electron with two Cu(II) nuclei (ΣI = 2 × 3/2) that leads to seven hyperfine splitting lines with $A_{||}$ about half of $A_{||}$ value for S = 1/2 Cu(II) species).[42]

The potentiometric titrations performed for Cu(II):L^1 system suggest the formation of four monomeric $[CuL^1H_n]$ (n = 1, 0, -1, -2) species (Table 2, Figure 1). The formation of the first complex, $[CuHL^1]^+$, starts already at pH around 2 (Figure 1). The UV–Vis absorption d–d band centered at 750 nm with ε = 35 $M^{-1}cm^{-1}$ (Figure S1, Table 2) indicates an involvement of one nitrogen atom besides oxygen donor(s) in copper ion binding. Potentiometry based stoichiometry suggests that one oxygen atom may come from the carboxylate unit while the other from phosphinate (if taking part in the coordination). EPR spectra with A_{II} = 160.7 and g_{II} = 2.30 further confirm the participation of one nitrogen in the binding (at pH below 2.5, before the coordination starts to take place, the EPR parameters: A_{II} = 119.0 G and g_{II} = 2.41, Figure S1, are in agreement with the Peisach-Blumberg plot for four oxygen donors[43]). Analogous EPR parameters were shown for the CuL complex in Cu(II):apl system.[20] In the case of no participation of the amino group in copper coordination, and binding only by oxygen donor(s), smaller A_{II} and larger g_{II} should be obtained, as observed for the Cu(II):appg CuL complex.[20] The deprotonation of $[CuHL^1]^+$ to $[CuL^1]$ is accompanied by a blue-shift of the d–d bands, with the Vis absorption maxima reaching 665 nm (Figure S1), indicating structural rearrangement and involvement of another nitrogen donor in copper coordination. Also, the change of EPR parameters, i.e. an increase of A_{II} to 175.3, and a decrease of g_{II} to 2.27 (Figure S2, Table 2) confirms the same set of donors. With further increase of pH, the next species, $[CuL^1H_{-1}]^-$, starts to dominate in solution, followed by the formation of $[CuL^1H_{-2}]^{2-}$. The minor changes in spectroscopic parameter, i.e. the d-d band centered at 645 nm (ε = 90 $M^{-1}cm^{-1}$) and EPR parameters (A_{II} = 175.3 and g_{II} = 2.27) correspond to two nitrogen atoms maintained in copper (II) coordination sphere. The negative number of protons in the notation of complexes means deprotonation of coordinated water molecules and the two species should be noted as $[CuL^1(OH)]^-$, and $[CuL^1(OH)_2]^{2-}$, respectively. Therefore, the tri- or quadri-dentate equatorial coordination of the L^1 ligand in the $[CuL^1]$ hampers the formation of CuL_2 species in the studied system.

Table 2. Potentiometric and spectroscopic data of Cu(II) complexes of studied ligands.[a]

	Potentiometry		UV-Vis		EPR		Reference
Species	log*β*	log*K*	λ_{max} [nm]	ε_{max}	A_{II}	g_{II}	
Cu(II) : L^1							This work
$[CuHL^1]^+$	23.24(5)		750	35	160.7	2.30	
$[CuL^1]$	19.37(4)	3.87	665	90	175.3	2.27	
$[CuL^1H_{-1}]^-$	10.05(5)	9.32	650	90	175.3	2.27	

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$[CuL^1H_{-2}]^{2-}$	-1.56(7)	11.61	650	90	Nd	Nd	
Cu(II) : L^2							This work
$[CuHL^2]^{2+}$	11.87(3)		730	50	Nd	Nd	
$[CuL^2]^{+}$	7.25(4)	4.62	700	75	Nd	Nd	
$[CuL^2_2]$	12.44(6)		665	125	165.4	2.28	
$[CuL^2_2H_{-2}]^{2-}$	-7.93(4)	20.28	Nd	nd	165.4	2.28	
Cu(II) : L^3							This work
$[CuL^3]$	11.55(7)		605	190	174.9	2.21	
Cu(II) : apl							20
$[CuHL]^{+}$	15.79		720	50	166	2.273	
$[CuL]$	12.08	3.71	667	61	181	2.248	
$[CuLH_{-1}]^{-}$	2.60	9.48	650	74	181	2.244	
$[CuL_2]^{2-}$	14.91						
Cu(II) : appg							20
$[CuHL]^{+}$	10.55		750	25	134	2.348	
$[CuL]$	5.66	4.89	720	30	134	2.330	
$[CuLH_{-1}]^{-}$	-1.46	7.12	683	39	136	2.295	
$[CuL_2]^{2-}$	9.55						
Cu(II) : MBAPA							35
$[CuL]$	9.87						
$[CuLH_{-1}]^{-}$	1.05	8.82					
$[CuLH_{-1}]^{2-}$	-9.48	10.53					
Cu(II) : AMPA							13
$[CuL]^{+}$	5.45		702	42	140	2.341	
$[CuL_2]$	9.99		670	61	135	2.293	
$[CuL_2H_{-1}]^{-}$	1.20	8.79	678	48	166	2.266	
Cu(II) : AM_2P							16
$[CuHL]^{2+}$	12.71						
$[CuL]^{+}$	7.64	5.07					
$[CuHL_2]^{+}$	19.79						
$[CuL_2]$	13.05	6.74	616				
$[CuL_2H_{-1}]^{-}$	3.07	9.98					
$[CuL_2H_{-2}]^{2-}$	-8.66	11.73					
Cu(II) : EDBPA							36
CuL	10.10						
Cu(II) : PDMBPA							37
CuL	3.18						
CuH_2L_2	17.79						
Cu(II) : PMPA							37
CuL	4.91						

[a] I = 0.1 M ($NaClO_4$), T = (25.0 ± 0.2) °C, solvent: H_2O for L^1 and L^3, and MeOH/H_2O 80:20 w/w for L^2.

UV–Vis experimental errors: λmax = ±2 nm, ε = ±5%.

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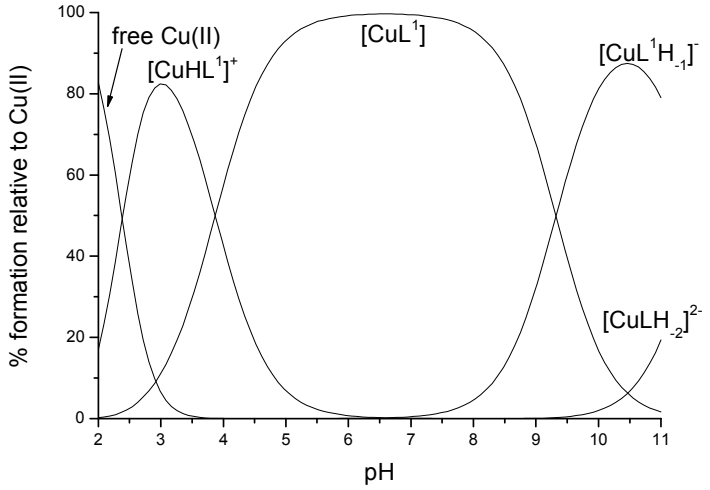
Because of the structural analogy, the Cu(II) complexes of L^1 were compared to apl and appg ligands, both possessing the aminocarboxylate function able to support Cu(II) coordination to aminophosphinate donor groups.[20] Both ligands formed protonated $[CuHL]^+$ complexes by coordinating the metal ion via the amino acid moiety, while the terminal NH_3^+ group was protonated. In the case of apl, this coordination resulted in the formation of a five-membered chelate ring with a N,O bonding mode, while in the case of appg only monodentate coordination of the carboxylate was possible at the pyroglutamate moiety; the tertiary amine was not able to bind the metal ion. The deprotonation of $[CuHL]^+$ was definitely accompanied by a structural rearrangement; in the [CuL] species, appg bound to Cu(II) at the aminophosphinate moiety, forming a five-membered N,O chelate ring, while apl coordinated in a tridentate manner via the phosphinate and the two amino groups, forming a (5,5)-membered joined chelate system. Quadridentate coordination of the apl ligand including the simultaneous binding of the carboxylate was also assumed. In contrast to Cu(II)-L^1 system, above pH 6, apl and appg formed $[CuL_2]^{2-}$ complexes.[20] The differences in the coordination ability of all three ligands are reflected in $\log K_{CuL}$ values (Table S2), indicating the advantageous arrangement of donors in L^1, with over 5 orders of magnitude gain in stability with respect to apl, and 13 to appg. In order to understand the impact of amine-phosphinate-Cu(II) binding in more detail, we compare the stability of Cu(II)- L^1 complexes with the apl and appg analogues on so-called competition plots, i.e. in a hypothetical situation in which equimolar concentrations of the three reagents (Cu(II), L^1 and apl, or appg, respectively) are mixed. Calculations are based on binding constants collected in Table 2, both for the new L^1 ligand and literature analogues.[20] From the competition plots presented in Figure 2, it is clear that the L^1 ligand is much more competitive towards Cu(II) binding than both, apl and appg ligands along entire pH range studied. The higher stability of L^1 complexes most probably stem from higher basicity of the carboxylate and tertiary amino groups, and their simultaneous involvement, together with aminophosphinate donors, in equatorial metal coordination in $[CuL^1]$, with the formation of three five-membered chelate rings.



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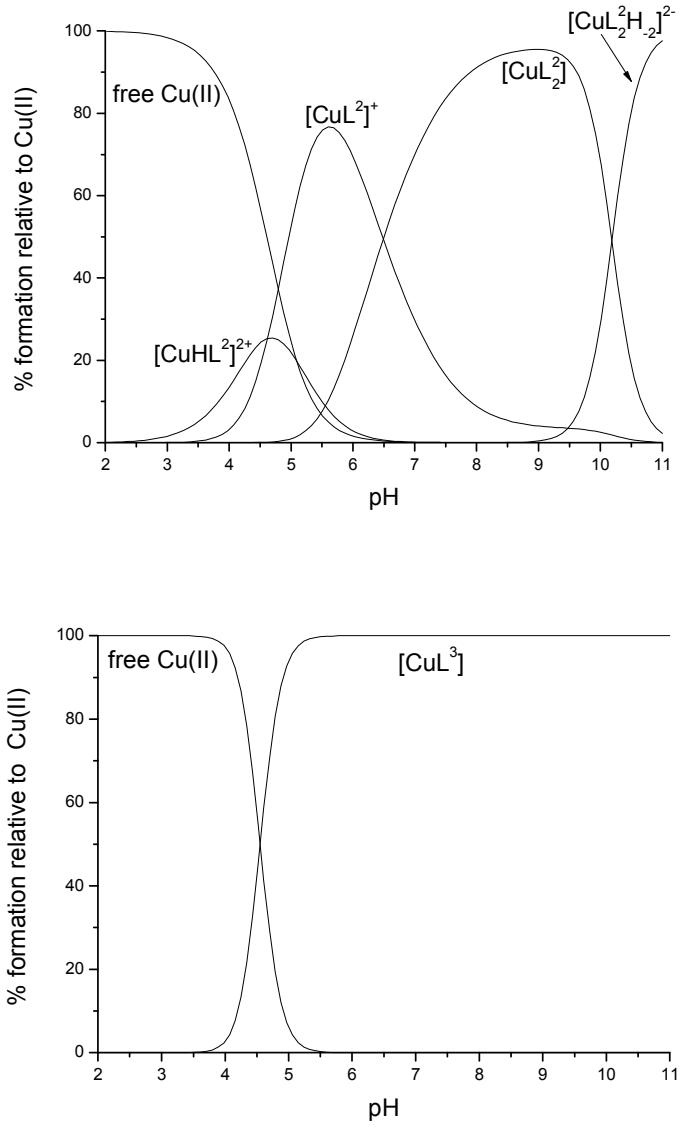

Figure 1. Species distribution profiles for Cu(II) complexes of L^1-L^3 in molar ratio Cu(II):L 1:2.

Data obtained for the second studied system, Cu(II):L^2, suggest that L^2 ligand might bind the metal ion in a tridentate manner, via primary amine, isoquinoline and phosphinate donor groups. The complexation starts with monomeric $[CuHL^2]^{2+}$ and $[CuL^2]^+$ species, which are present in solution in pH range of 3 - 8.5 (with the maximum at pH 4.7 and 5.6, respectively, Figure 1). The precipitation of bluish precipitate above pH around 7 at Cu(II) to L^2 ratio of 1:1 prevented the detection of $[CuL^2H_{-1}]$ species. The monomeric complexes are followed by $[CuL^2_2]$ species, prevailing in pH range of 7-10. Besides these three species, the formation of a hydroxo $[CuL^2_2H_{-2}]^{2-}$ (or more precisely $[CuL^2_2(OH)_2]^{2-}$ was calculated as a further base-consuming process took place over pH 10. The species corresponds to simultaneous deprotonation of two water molecules in the copper (II) coordination sphere. The

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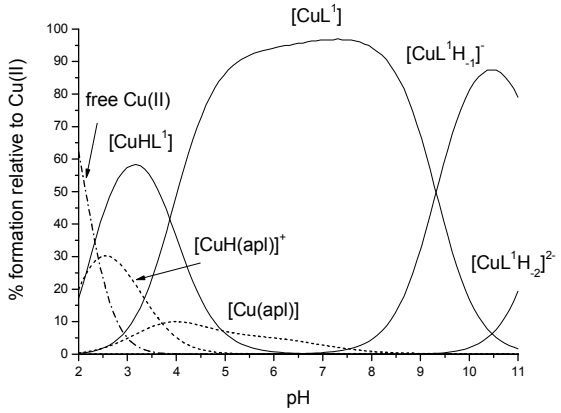
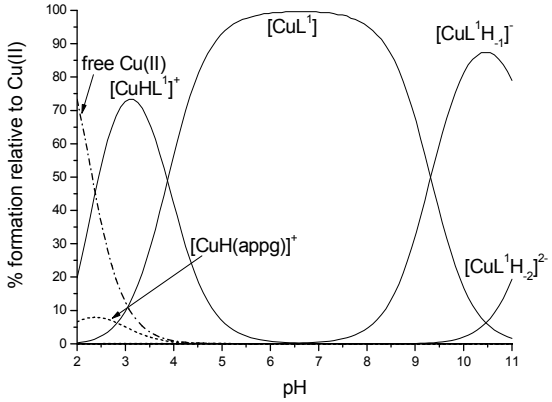
complex formation scheme corresponds to Cu(II) binding process described previously for aminophosphinates.[13, 16]

The UV-Vis and EPR spectral parameters are consistent with the speciation model based on the potentiometric data and proposed binding modes. In the UV-Vis spectra collected over pH 4 - 5.5, the maximum of d-d band shifts from 740 nm (ε = 50 $M^{-1}cm^{-1}$) towards 700 nm (ε = 75 $M^{-1}cm^{-1}$), respectively. Although d-d band with maximum at 700 nm is rather indicative for 1N coordination, it is sensitive mainly to the equatorial coordination and the observed wavelength does not exclude coordination of the second nitrogen in the axial position in $[CuL^2]^+$ complex. The logK between the two monomeric $[CuHL^2]^{2+}$ and $[CuL^2]^+$ species is 0.84 log*K* units lower than the constant of $N_{Isoquinoline}$ in free ligand (4.62 *versus* 5.46, Table 1), what may suggest its involvement in Cu(II) binding in $[CuL^2]^+$ (together with other two donor atoms). The formation of $[CuL^2{}_2]$ is reflected by the shift of the UV-Vis bands towards 665 nm (ε = 125 $M^{-1}cm^{-1}$) (Table 2, Figure S1), and this spectral behaviour is characteristic for the presence of two nitrogen donors in complex coordination sphere, here from two aminophosphinate moieties. The lack of any further significant changes in visible spectra confirms that the coordination behaviour of $[CuL^2{}_2H_{-2}]^{2-}$ complex does not change. EPR parameters, A_{II}=165.4 and g_{II}=2.28 are typical for 2N species with square-bypiramidal geometry[13] (Figure S2, Table 2), indicating aminophosphinate mode of binding.

Because of possible coordination patterns of L^2 ligand, i.e. tridentate via primary amine, isoquinoline and phosphinate donor groups in $[CuL^2]^+$ species, and bidentate through aminophosphinate moieties in $[CuL^2{}_2]$ and $[CuL^2{}_2H_{-2}]^{2-}$ complexes, we compare its binding ability with the AM_2P[16] and AMPA[13] chelators. Although an increase of L^2 logK_{CuL} and logK_{CuL2} (Table S2) may indicate that it forms stronger copper complexes than the AMPA analogue,[13] yet they are weaker than the species formed by AM_2P.[16] However, it has to be underlined, that current thermodynamic data have been obtained in a methanol/water mixture (80:20 w/w), and previously a high amount of methanol in solvent composition was shown to increase the stability constants of Cu(II):aminohydroxamate complexes by several orders of magnitude.[38, 44, 45] Therefore, it can be concluded that the stabilization of the Cu(II):L^2 complexes may come from the intrinsic property of the ligand and complexes structures, supported by subtle effects of organic solvent, like specific solvation effects, or electric permeability.





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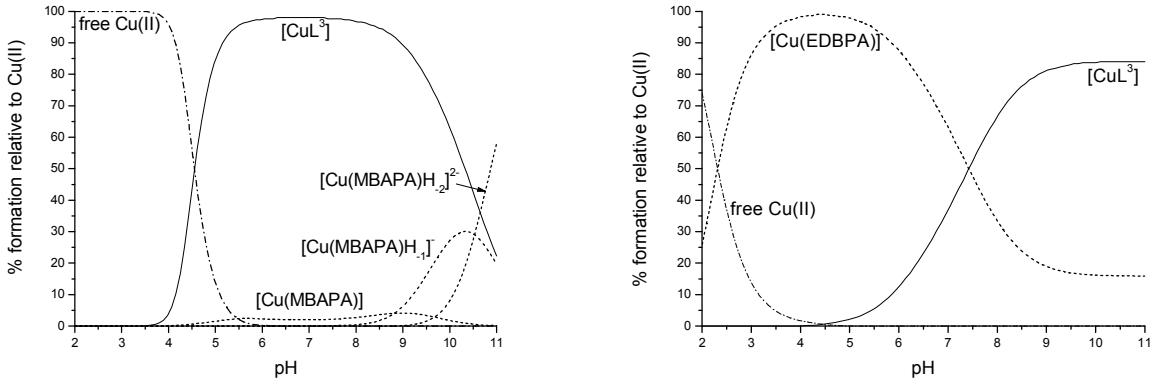

Figure 2. Competition plots between studied ligands, their corresponding analogues and Cu(II), describes complex formation at different pH values in a hypothetical situation in which equimolar amounts of the three reagents are mixed. Calculations are based on binding constants from Table 2. Conditions: [Cu(II)] = [L^{1-3}] = [L] = 3 mM.

The potentiometric data for Cu(II):L^3 system indicate the formation of only one, monomeric, species [CuL^3], present in solution above pH 4.5 (Table 2, Figure 1). The protonated and hydroxo species introduced into calculated model were rejected. The absorption spectra show d-d transitions maximum centered at 605 nm (ε = 190 $M^{-1}cm^{-1}$) (Figure S1, Table 2), even when pH-dependent titrations were carried out for Cu(II):L^3 molar ratio 1:2. The EPR parameters also indicate the formation of only one complex with parameters A_{II} = 174.9 and g_{II} = 2.21. These spectroscopic parameters are about the usual characteristics of 3N species,[38, 43] and therefore may suggest the involvement of three nitrogen atoms (two from primary amines and one from piperazine ring), and one or two phosphinate oxygens in Cu(II) coordination environment. The lack of protonated species further supports this coordination environment. For 2:1 Cu(II):L^3 molar ratio, despite excess of the Cu(II) used, no species of M_2L stoichiometry could be identified , and a blueish precipitate occurred above pH 6 (this pH range was not included in the data processing). Earlier studies carried out for PDMBPA showed the formation of CuL and $Cu(HL)_2$ complexes, with only one side of the ligand involved in metal binding via the piperidine nitrogen and phosphinic oxygen.[37] For this ligand the possibility of the inversion of the piperazine ring to *gauche* conformation and simultaneous coordination of both piperazine N atoms to Cu(II) could not be assumed due to a rigidity of the piperazine ring. Moreover, the complexes formed were less stable than those for the PMPA (Scheme 1).[37] However, the comparison of the coordination ability of L^3 to these two ligands should be restricted to pH 7, as above a formation of insoluble polymeric species was assumed, for which stability constants could not be determined. An efficient combination of amino and phosphinic groups has been proposed in MBAPA,[35] and EDBPA,[36] which revealed strong, tetradentate complexation of Cu(II) ions via both phosphinate oxygen atoms and both amine groups ($\log K_{CuL}$ of 9.49[35] and 10.10,[36] respectively). The chelating efficiency of discussed L^3 ligand, reflected as $\log K_{CuL}$ = 11.55, exceeds the ones corresponding to the above bis(amino-phosphinic) ligands by 1.5-2 orders of magnitude, what suggests an involvement of an additional factor influencing the stability. Therefore, the proposed arrangement of donors, i.e. location of the amino group close to the phosphinate and piperazine atoms, enhances the binding power of the ligand, making it an efficient chelator. The competition results shown in Figure 2 clearly indicate that L^3 ligand forms much more stable

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complexes than the MBAPA along entire pH range. However, the competition between L^3 and EDBPA chelator,[36] shows that for the latter system, [CuL] complex predominates the solution already from pH 2.5, while [CuL^3] is formed only above pH 4.5, and become significantly more competitive above pH 7. We can hypothesize that this additional stability of L^3 at alkaline pH might be explained by the participation of all donor groups in metal coordination and the formation of five five-membered chelate rings with completion of Cu(II) coordination sphere by one ligand. It's difficult to assume if there is an inversion of the piperazine ring in L^3, but such a rearrangement would be necessary for the formation of 3N or 4N Cu(II) complex.

4. Conclusions

The three new non-symmetrical bis(aminoalkyl)phosphinate ligands, namely L^1–L^3, were synthesized and evaluated for coordination of Cu(II) ions. The protonation schemes of the ligands followed classical scheme, with logK constants of amine, piperazine, carboxylate and phosphinate groups being in a good agreement with corresponding values reported earlier for analogous compounds. For all ligands the metal-to-ligand stoichiometry of Cu(II) complexes was determined by ESI-MS, and further confirmed by potentiometric studies. Potentiometric titrations together with a full spectroscopic analysis clarified the species distribution profiles and detailed coordination modes. The results show that all ligands are good chelating agents for Cu(II) ions, with L^1 and L^3 being especially efficient. The arrangement of donors in L^1 ligand allows its wrapping around Cu(II) ions in [CuL^1] complex, making the complexation very competitive. Although it's difficult to assume if there is an inversion of the piperazine ring in L^3 facilitating formation of 3N or 4N [CuL^3] complex, it is clear that the addition of amino groups to piperazine-1,4-diylbis(methylene)bis(phosphinic acid), enhanced the binding power of the ligand, making it an efficient chelator.

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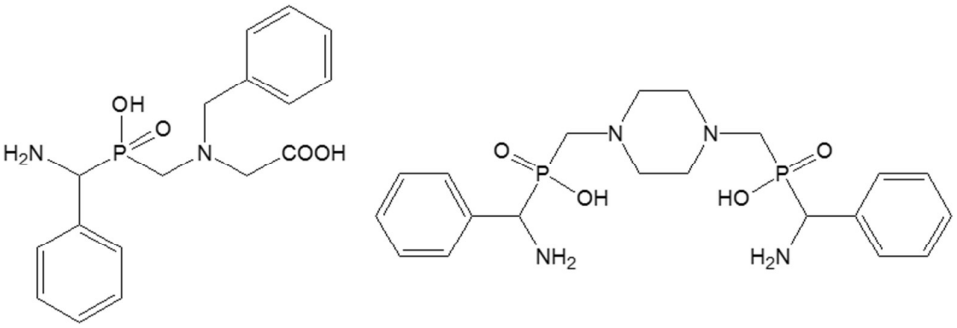

Novel, non-symmetrical bis(aminoalkyl)phosphinic acids exhibit enhanced efficiency in Cu(II) ions binding.