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Synthesis of *N*-methyl alkylaminomethane-1,1-diphosphonic acids and evaluation of their complex-formation abilities towards copper(II)

Barbara Kurzak [a,*], Waldemar Goldeman [b], Magdalena Szpak [a], Ewa Matczak-Jon [b], Anna Kamecka [c]

[a] Faculty of Chemistry, University of Opole, Oleska 48, 45-052 Opole, Poland
[b] Department of Chemistry, Wrocław University of Technology, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland
[c] Institute of Chemistry, Siedlce University of Natural Sciences and Humanities, 3 Maja 54, 08-110 Siedlce, Poland

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ABSTRACT

A series of *N*-methyl alkylaminomethane-1,1-diphosphonic acids (**3a**–**g**) with a common tertiary nitrogen atom (CH_3–N–R) bearing linear or branched alkyl, cycloheptyl or phenylalkyl R substituents was synthesized in the reaction of diethylphosphonate with triethyl orthoformate and secondary amine followed by hydrolysis, and by the Eschweiler–Clarke methylation of the alkylaminomethane-1,1-diphosphonic acids with formic acid and formaldehyde. Complex-formation abilities of **3a**–**g** towards copper(II) in solution were studied by means of pH-potentiometry, ESI-MS spectrometry, UV–Vis and EPR methods.

Evaluation of stability constants for the Cu(II) complexes of **3a**–**g** has revealed their dependence on a combination of steric and electronic effects imposed by R substituents. It has been demonstrated that compounds with linear or branched alkyl on the N atom (**3a**, **3d**, **3e**) form the most stable complexes with Cu(II), the least stable are complexes of **3f**, which can be attributed to the steric effect imposed by cycloheptyl ring attached directly to the N atom. In addition to the main $[Cu(HL)_2]$ complexes, with well-known {O, O} chelating binding mode, [Cu(HL)L] and $[CuL_2]$ species with the {O, N} donor set, protonated dinuclear $[Cu_2H_xL_3]$ ($x = 4, 5, 6$) species have been detected in all studied systems. The nitrogen coordination in the [Cu(HL)L] and $[CuL_2]$ complex has been confirmed by Vis and EPR spectra.

1. Introduction

Bisphosphonates, a class of compounds sharing a common P–C–P backbone, are known to play a crucial role in various medical applications. At now, they are considered the most effective drugs for prevention and treatment of bone disorders such as osteoporosis, Paget's disease and osteolytic bone metastases [1–4]. The medical use of bisphosphonates stems from their specific properties such as strong affinity of phosphonic/phosphonate groups for various metal ions as well as their high potential to form hydrogen bonds [5,6]. It is widely accepted that antiresorptive properties of bisphosphonates rely on two mechanisms: (a) high affinity for calcium ions enables their targeting to mineral component of bone (hydroxyapatite) that prevents both its growth and dissolution/breakdown (b) after absorption on bone surface they target selectively bone resorbing cells (osteoclasts) and inhibit their activity. It is well documented that the major target of the most potent bisphosphonates characterized by the presence of one of more nitrogen atoms in their side chains (NBPs) is magnesium-dependent enzyme of the mevalonate pathway, farnesyl pyrophosphate synthase (FPPS) [7–9].

A high affinity of bisphosphonates for hydroxyapatite has also been utilized to deliver diagnostic and therapeutic pharmaceuticals to bone surface [10–12]. Moreover, some bisphosphonate-functionalized compounds have been developed as potential chelating agents in metal intoxication therapy [13], and some others have proven to be useful reagents for detecting biologically important metal ions [14]. On the other hand, complexation of metal ions, followed by spectrophotometric detection of M(II) complexes (M = Cu, Fe) has been shown to be useful approach for quantification of bisphosphonates in biological fluids and pharmaceutical formulations [15–17]. Considering a broad spectrum of biological utilities of bisphosphonates as well as their strong affinity for metal ions, detailed structure-correlated studies of individual properties of ligands and in-depth understanding complex-formation driving factors, thermodynamic stability of metal complexes and their speciation in solution are desired. This might help to establish structure-activity relationships and shed light on the *in vivo* mode of action of bisphosphonate-based compounds.

Bisphosphonates display unique coordination properties accounted for by a short distance between two phosphonate

* Corresponding author.
E-mail address: bkurzak@gmail.com (B. Kurzak).

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functions, which facilitates their synergic ability of acting as one [CP_2O_6] unit, which affords involvement up to six phosphonate O-donors in metal ions coordination. This results in a variety of coordination modes, depending on a size and chemical nature of side chains attached to the carbon atom, protonation degree of phosphonic/phosphonate groups, specificity and coordination geometry requirements of a given metal ion as well as presence/absence of additional functional groups in their structures [5]. The studies on the Zn(II), Mg(II) and Ca(II) complexation by structurally diverse derivatives of aminomethane-1,1-diphosphonic acid characterized by the presence of C_α–N_{amino} bond (Scheme 1, left) have revealed several key feature that seem to be universal when considering their complex-formation abilities in solution [18–21]. Despite the incorporation of the amino functionality, compounds of this class demonstrate preference towards chelation *via* the phosphonate functions and formation of O-bonded complexes over a broad range of pH. This is because of strong basicity of the nitrogen proton that permits the formation of nitrogen-bonded complexes only in alkaline solutions. Indeed, tendency towards formation of soluble, protonated oligonuclear complexes has been observed, which is especially notable at 1:1 metal to ligand ratio, in the intermediate pH range and at higher concentrations. Some aminomethane-1,1-diphosphonic acid derivatives have also been suggested to form oligonuclear complexes with Cu(II) [22,23].

Herein we describe the synthesis of *N*-methyl alkylaminomethane-1,1-diphosphonic acids (Scheme 1, right) with common tertiary nitrogen atom (CH_3)–N–R, where R is linear or branched alkyl, cycloheptyl or phenylalkyl as well as their complex-formation abilities towards copper(II) in solution studied by means of pH-potentiometry, ESI-MS, EPR and NIR–Vis–UV methods. Compounds **3a**–**g** were designed with an intention to obtain aminobisphosphonates with improved solubility in water as compared to previously studied mono *N*-substituted compounds (Scheme 1, left).

Searching data bases we found only few examples of *N*-methyl alkylaminomethane-1,1-diphosphonic acids, like *N*-octyl and *N*-hexyl [24], *N*-(2-carboxylethyl) [25], *N*-cycloheptyl (*N*-methyl *Incadronate*) [26], *N*-benzyl derivative [27,28], and the simplest one, namely *N*,*N*-dimethylaminomethane-1,1-diphosphonic acid, the only one which has been extensively studied. Some biological [29,30] and NMR [31,32], studies of *N*,*N*-dimethylaminomethane-1,1-diphosphonic acid have been performed, and solid state studies have revealed diversified coordination capabilities of such ligand affording the formation of metal complexes with a whole spectrum of structures, from 0D isolated coordination units up to 3D coordination frameworks [33–37]. Moreover, complexation abilities of *N*,*N*-dimethylaminomethane-1,1-diphosphonic acid have been examined in solution [38,39], indicating the formation of highly stable mononuclear complexes with trivalent metal ions such as Ga^{3+}, In^{3+}, and Fe^{3+}.

2. Experimental

2.1. General information

The $^{31}P\{^1H\}$, $^{13}C\{^1H\}$ and ^1H NMR spectra were recorded on a Bruker DRX spectrometer operating at 121.50 MHz, 75.46 MHz and 300.13 MHz, respectively, at 300 K, and are given in relation to 85% H_3PO_4 (^{31}P NMR) and $SiMe_4$, (^1H, ^{13}C). All experiments were performed with locking spectrometer on deuterium from a solvent.

All reagents were of commercial quality, purchased from Sigma–Aldrich and were used without further purification. The identity of synthesized compounds was confirmed by NMR, their purity, the concentrations of the ligand stock solutions used for potentiometric measurements and the HCl concentration were determined by pH-potentiometric titrations using the Gran's method [40]. The $CuCl_2$ and HCl stock solutions were prepared from Titrisol concentrates (Merck) for the preparation of standard solutions. The exact concentration of copper ions stock solution was determined *via* complexometric ethylenediaminetetraacetate (EDTA) titration. Carbonate-free potassium hydroxide solution (the titrant) was prepared from cc. KOH and standardized against a standard potassium hydrogen phthalate solution. All solutions were prepared with bi-distilled water.

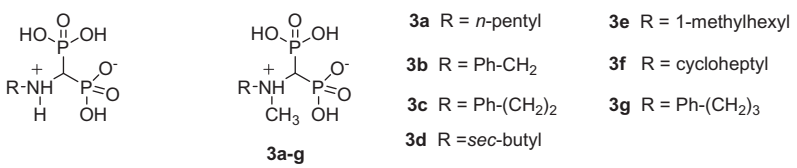
2.2. Syntheses

N-Methyl-*n*-pentylamine was prepared from *n*-pentylamine according to literature procedure with 53% yield (bp_{760} = 114–118 °C) [41]. 3-Phenylpropyl isocyanide was prepared from 3-phenylpropylamine by standard procedure [42], and the crude product was purified by bulb to bulb distillation with 44% yield (bp_{20} = 140–150 °C). The NMR spectra of obtained 3-phenylpropyl isocyanide are consistent with given in the literature [43].

2.2.1. Synthesis of N-methyl alkylaminomethane-1,1-diphosphonic acid ***3a*** and ***3b***

A mixture of *N*-methylbenzylamine (12.1 g, 0.10 mol) or *N*-methyl-*n*-pentylamine (10.1 g, 0.10 mol), diethyl phosphonate (27.6 g, 0.20 mol) and triethyl orthoformate (16.3 g, 0.11 mol) was stirred and heated at about 150–155 °C (oil bath temperature) with continuous distillation of the ethanol produced. When the evolution of ethanol has cased (about 4 h), the reaction mixture was cooled to room temperature, the volatiles were removed by rotary evaporation (boiling water bath, 20 mmHg) and the residue was treated with 6 M hydrochloric acid (300 ml). Obtained solution was heated under reflux for 8 h, the hydrolysate was cooled to room temperature and washed with dichloromethane (1 × 50 ml, 3 × 20 ml). Water phase was evaporated to dryness (boiling water bath, 20 mmHg) and repeatedly co-distilled with water (5 × 100 ml). Obtained crude product was treated with acetone (50 ml), filtered off, washed with acetone (4 × 25 ml) and dried in air, yielding **3a** (10.6 g, 36% yield, based on *N*-methylbenzylamine) and **3b** (14.3 g, 52% yield, based on *N*-methyl-*n*-pentylamine) as white solids. Crude solids were purified by recrystallization from water solutions.

N-Methylpentylaminomethane-1,1-diphosphonic acid (**3a**): $^{31}P\{^1H\}$(121 MHz, D_2O+NaOD) δ: 9.63 (s); ^1H NMR (300 MHz, D_2O+NaOD) δ: 0.73 (t, 3H, J = 6.8 Hz, CH_3), 1.10–1.30 (m, 4H, $CH_3(CH_2)_2$), 1.52 (quintet, 2H, J = 7.7 Hz, NCH_2CH_2), 2.87 (s, 3H, CH_3N), 3.02 (t, 1H, J = 19.1 Hz, CHP_2), 3.23 (t, 2H, J = 7.7 Hz, NCH_2); $^{13}C\{^1H\}$NMR (75 Hz, D_2O+NaOD) δ: 13.10 (CH_3), 21.57 (CH_2), 24.79 (CH_2), 27.66 (CH_2), 40.24 (NCH_3), 57.01 (NCH_2), 63.12 (t, J = 114.4 Hz, CHP_2).



Scheme 1. Mono *N*-substituted (left) and *N*-methyl alkylaminomethane-1,1-diphosphonic acids **3a**–**g** (right).

N-Methylbenzylaminomethane-1,1-diphosphonic acid (**3b**): $^{31}P\{^1H\}$(121 MHz, D_2O+NaOD) δ: 8.35 (s); ^1H NMR (300 MHz, D_2O+NaOD) δ: 2.84 (s, 3H, CH_3N), 3.18 (t, 1H, J = 18.6 Hz, CHP_2), 4.54 (s, 2H, CH_2), 7.37–7.40 (m, 3H, ArH), 7.42–7.47 (m, 2H, ArH); ^{13}C NMR (75 Hz, DMSO-d_6) δ: 41.56 (NCH_3), 57.68 (t, J = 118.6 Hz, CHP_2), 59.67 (NCH_2), 129.39, 130.01, 131.40, 131.52.

2.2.2. Synthesis of alkylaminomethane-1,1-diphosphonic acid 3c′-f′

Compounds **3c′**–**f′** were obtained according to the same procedure as **3a** and **3b** except that instead of *N*-methylbenzylamine and *N*-methyl-*n*-pentylamine, a phenethylamine (12.1 g, 0.10 mol), (±)-*sec*-butylamine (7.3 g, 0.10 mol), (±)-2-heptylamine (11.5 g, 0.10 mol) and cycloheptylamine (11.3 g, 0.10 mol) were used. The crude products were crystallized from: water–acetone mixture (1:2, v/v) in a case of **3c′**, yields 13.3 g (45%, based on phenethylamine), water–96% ethanol mixture (1:2, v/v) in a case of **3d′**, yields 6.2 g (25%, based on (±)-*sec*-butylamine), water in a case of **3e′**, yields 12.1 g (42%, based on (±)-2-heptylamine) and water–acetone mixture (1:1, v/v) in a case of **3f′**, yields 16.6 g (58%, based on cycloheptylamine).

2-Phenylethylaminomethane-1,1-diphosphonic acid (**3c′**): ^{31}P NMR $\{^1H\}$ (121 MHz, D_2O+NaOD): δ 18.02; ^{1}HNMR (300 MHz, D_2O+NaOD): δ 2.32 (t, J = 17.2 Hz, 1H, CHP_2) 2.53 (t, J = 7.6 Hz, 2H, CH_2), 2.79 (t, J = 7.6 Hz, 2H, CH_2), 7.00 (m, 1H, p-ArH), 7.07–7.14 (m, 4H, m- and o-ArH); ^{13}CNMR (300 MHz, D_2O+NaOD): δ 35.63 (CH_2), 52.03 (bs, CH_2), 59.070 (t, J = 128.9 Hz, NCH), 126.00, 128.48, 128.63, 140.48.

(±)-1-Methylpropylaminomethane-1,1-diphosphonic acid (**3d′**): $^{31}P\{^1H\}$(121 MHz, D_2O+NaOD) δ: 8.78 (s, I = 100%), 8.90 (s, I = 100%); ^1H NMR (300 MHz, D_2O+NaOD) δ: 0.81 (t, 3H, J = 7.5 Hz, CH_3CH_2), 1.09 (d, 3H, J = 6.4 Hz, CH_3CH), 1.37 (m, 1H, CH_aH_b), 1.61 (m, 1H, CH_aH_b), 2.90 (t, 1H, J = 16.5 Hz, CHP_2), 3.34 (m, 1H, $NCHCH_3$); $^{13}C\{^1H\}$NMR (75 Hz, D_2O+NaOD) δ: 9.10 (CH_3), 16.95 (CH_3), 27.27 (CH_2), 54.51 (bs, NCH), 55.30 (t, J = 123.4 Hz, CHP_2).

(±)-1-Methylhexylaminomethane-1,1-diphosphonic acid (**3e′**): $^{31}P\{^1H\}$(121 MHz, D_2O+NaOD) δ: $^{31}P\{^1H\}$(121 MHz, D_2O+NaOD) δ: 18.00 (d, J = 4.5 Hz, I = 100%), 17.88 (d, J = 4.5 Hz, I = 100%); ^1H NMR (300 MHz, D_2O+NaOD) δ: 0.62 (t, 3H, J = 6.5 Hz, CH_3CH_2), 0.79 (d, 3H, J = 6.2 Hz, CH_3CH), 0.91–1.24 (m, 7H, 3 × CH_2 + CH_aH_b),1.36 (m, 1H, CH_aH_b), 2.60 (t, 1H, J = 17.5 Hz, CHP_2), 2.87 (m, 1H, $NCHCH_3$); $^{13}C\{^1H\}$NMR (75 Hz, D_2O+NaOD) δ: 13.26 (CH_3), 15.76 (CH_3), 21.71 (CH_2), 24.08 (CH_2), 30.73 (CH_2), 32.81 (CH_2), 53.61 (t, J = 112.6 Hz, CHP_2), 56.52 (bs, NCH).

Cycloheptylaminomethane-1,1-diphosphonic acid (**3f′**, *Incadronate*): $^{31}P\{^1H\}$(121 MHz, D_2O+NaOD) δ: 8.50 (s); ^1H NMR (300 MHz, D_2O+NaOD) δ: 1.35–1.43 (m, 6H, 2 × CH_2 + 2 × CH_aH_b), 1.53–1.67 (m, 4H, 2 × CH_aH_b + 2 × CH_cH_d), 2.03 (m, 2H, 2 × CH_cH_d), 3.41 (t, 1H, J = 18.0 Hz, CHP_2), 3.58 (m, 1H, NCH,); $^{13}C\{^1H\}$NMR (75 Hz, D_2O+NaOD) δ: 23.80 (CH_2), 27.86 (CH_2), 33.15 (CH_2), 55.32 (t, J = 127.6 Hz, CHP_2), 67.68 (t, J = 4.8 Hz, CHN). The ^1H NMR spectrum is consistent with literature data [26].

2.2.3. Synthesis of 3-phenylpropylaminomethane-1,1-diphosphonic acid 3g′

Bisphosphonic acid **3g′** was prepared according to the methodology developed previously in our laboratory [44], starting from 3-phenylpropylisocyanide (7.3 g, 0.05 mol) and yields 13.1 g of 3-phenylpropylaminomethane-1,1-diphosphonic **3g′** (85%, based on starting isocyanide, see Scheme 2).

3-Phenylpropylaminomethane-1,1-diphosphonic acid (**3g′**): ^{31}P $\{^1H\}$ (121 MHz, D_2O+NaOD) δ: 18.33 (s); ^1H NMR (300 MHz, D_2O+NaOD) δ: 1.40 (quintet, 2H, J = 7.4 Hz, CH_2), 2.20 (t, 1H, J = 17.1 Hz, CHP_2), 2.30 (t, 2H, J = 7.4 Hz, CH_2), 2.45 (t, 2H, J = 7.4 Hz, CH_2), 6.87–7.03 (m, 5H, Ph); ^{13}C NMR (75 Hz, D_2O+NaOD) δ: 31.49 (CH_2), 32.90 (CH_2), 50.73 (t, J = 5.6 Hz, CH_2N), 59.44 (t, J = 129.5 Hz, CHP_2), 125.77, 128.41, 128.49,142.93.

2.2.4. Synthesis of N-methyl alkylaminomethane-1,1-diphosphonic acids 3c-g

To suspension of alkylaminomethane-1,1-diphosphonic acids **3c′**–**g′** (0.010 mol) in water (10 ml), formic acid (50 ml) and 36% solution of formaldehyde in water (4.2 g, 0.05 mol) were added and the reaction mixture was heated at reflux for 24 h. The reaction mixture was cooled to room temperature and evaporated to dryness (boiling water bath, 20 mmHg) and repeatedly co-distilled with water (5 × 50 ml). Obtained residue was treated with acetone (20 ml), filtered off, washed with acetone (4 × 10 ml) and dried in air, yielding the crude products: 2.5 g of **3c** (80%), 2.3 g of **3d** (90%), 2.4 g of **3e** (81%), 2.5 g of **3c** (83%) and 3.1 g of **3g** (97%) as white solids. The crude products were crystallized from water–96% ethanol mixture (1:1, v/v) in a case of **3c** and **3f**, and from water solutions in case of **3d**, **3e** and **3g**.

N-Methyl-2-phenylethylaminomethane-1,1-diphosphonic acid (**3c**): ^{31}P $\{^1H\}$(121 MHz, D_2O+NaOD) δ: 16.28 (s); ^1H NMR (300 MHz, D_2O+NaOD) δ: 2.42 (s, 3H, CH_3), 2.56 (m, 2H, CH_2, AA′BB′ system), 2.74 (t, 1H, J = 21.8 Hz, CHP_2), 2.88 (m, 2H, NCH_2, AA′BB′ system), 7.00 (m, 1H, J = 4.3 Hz (virtual coupling), p-ArH), 7.08 (d, 4H, J = 4.3 Hz (virtual coupling), m- and o-ArH); $^{13}C\{^1H\}$NMR (75 Hz, D_2O+NaOD) δ: 33.33 (CH_2), 40.05 (CH_3), 58.20 (CH_2), 64.69 (t, J = 120.6 Hz, CHP_2), 126.29, 128.63, 128.88, 139.71.

(±)-*N*-Methyl-1-methylpropylaminomethane-1,1-diphosphonic acid (**3d**): $^{31}P\{^1H\}$(121 MHz, D_2O+NaOD) δ: 8.83 (s, I = 100%), 8.95 (s, I = 100%); ^1H NMR (300 MHz, D_2O+NaOD) δ: 0.77 (t, 3H, J = 7.4 Hz, CH_3CH_2), 1.16 (d, 3H, J = 6.5 Hz, CH_3CH), 1.48 (m, 1H, CH_aH_b), 1.67 (m, 1H, CH_aH_b), 2.90 (s, 3H, CH_3N), 3.24 (t, 1H, J = 19.1 Hz, CHP_2), 3.68 (m, 1H, $NCHCH_3$); $^{13}C\{^1H\}$NMR (75 Hz, D_2O+NaOD) δ: 8.85 (CH_3), 14.11 (CH_3), 24.43 (CH_2), 36.47 (NCH_3), 59.40 (t, J = 108.0 Hz, CHP_2), 64.64 (NCH).

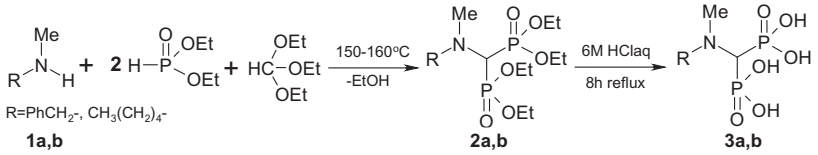
(±)-*N*-Methyl-1-methylhexylaminomethane-1,1-diphosphonic acid (**3e**): $^{31}P\{^1H\}$(121 MHz, D_2O+NaOD) δ: $^{31}P\{^1H\}$(121 MHz, D_2O+NaOD) δ: 9.53 (s, I = 100%), 9.91 (s, I = 100%); ^1H NMR (300 MHz, D_2O+NaOD) δ: 0.64 (t, 3H, J = 6.7 Hz, CH_3CH_2), 1.05–1.25 (m, 9H, 3 × CH_2 + CH_3CH), 1.32 (m, 1H, CH_aH_b), 1.60 (m, 1H, CH_aH_b), 2.81 (s, 3H, CH_3N), 3.18 (t, 1H, J = 19.3 Hz, CHP_2), 3.59 (m, 1H, $NCHCH_3$); $^{13}C\{^1H\}$NMR (75 Hz, D_2O+NaOD) δ: 13.24 (CH_3), 15.48 (CH_3), 21.72 (CH_2), 23.66 (CH_2), 30.89 (CH_2), 31.60 (CH_2), 36.74 (NCH_3), 61.88 (t, J = 114.0 Hz, CHP_2), 62.19 (t, J = 2.3 Hz, NCH).

N-Methylcycloheptylaminomethane-1,1-diphosphonic acid (**3f**, *N-methyl Incadronate*): ^{31}P $\{^1H\}$ (121 MHz, D_2O+NaOD) δ: 8.80 (s); ^1H NMR (300 MHz, D_2O+NaOD) δ: 1.45–1.65 (m, 8H, 4 × CH_2), 1.77 (m, 2H, 2 × CH_aH_b), 2.07 (m, 2H, 2 × CH_aH_b), 3.03 (s, 3H, CH_3), 3.37 (t, 1H, J = 19.0 Hz, CHP_2), 3.89 (m, 1H, NCH,); ^{13}C $\{^1H\}$NMR (75 Hz, D_2O+NaOD) δ: 23.39 (CH_2), 27.05 (CH_2), 29.17 (bs, CH_2), 36.61 (CH_3), 60.48 (t, J = 108.8 Hz, CHP_2), 67.68 (s, CHN).

N-Methyl-3-phenylpropylaminomethane-1,1-diphosphonic acid (**3g**): ^{31}P $\{^1H\}$ (121 MHz, D_2O+NaOD) δ: 13.27 (s); ^1H NMR (300 MHz, D_2O+NaOD) δ: 1.62–1.72 (m, 2H, CH_2CH_2N), 2.43 (bt, 2H, J = 7.7 Hz, CH_2), 2.57 (s, 3H, CH_3), 2.84 (t, 1H, J = 20.5 Hz, CHP_2), 2.93 (m, 2H, NCH_2, AA′BB′ system), 7.04 (m, 1H, p-ArH), 7.09–7.16 (m, 4H, m- and o-ArH); ^{13}C $\{^1H\}$NMR (75 Hz, D_2O+NaOD) δ: 28.41 (CH_2), 32.40 (CH_2), 40.05 (CH_3), 56.34 (CH_2), 64.28 (t, J = 111.5 Hz, CHP_2), 125.98, 128.44, 128.55, 142.15.

2.3. Potentiometric measurements

The potentiometric titrations were performed in 3 cm^3 samples with the ligand concentration of 1.5×10^{-3} mol dm^{-3}. Potentiometric measurements of the free ligands were carried out in the range pH 2–11.5. The copper(II)-to-ligand molar ratios ranged from 1:1.5 to 1:3, three or four different ratios were applied for each system studied. These samples were titrated in the pH range 2.5–11.5.



Scheme 2. Direct synthesis of *N*-methyl alkylaminomethane-1,1-diphosphonic acids **3a** and **3b**.

Precipitation was observed at pH values above 4 when metal: ligand molar ratio was <1:1.5 and in the systems with metal excess.

The potentiometric titrations were performed using an automatic titrator system Titrando 809 (Metrohm) with a Metrohm 6.0234.100 type combined glass electrode filled with 3 M KCl in water. The measurements were carried out in an argon atmosphere at 298 K at constant ionic strength (0.2 mol dm^{-3} KCl). The electrode system was calibrated daily in hydrogen ion concentration using HCl solution (0.02 mol dm^{-3} in KCl) against standard KOH solution (0.15 mol dm^{-3}). The resulting titration data were used to calculate the standard electrode potentials, $E°$, and the dissociation constant for water (pK_w = 13.74 ± 0.02). The calibration of the electrode was as pH = –log[H^+].

The protonation constants of the ligand and the concentration stability constants ($\log\beta_{pqr}$) of metal complexes were calculated by means of a general computational program, HYPERQUAD 2006 [45]. The uncertainties (3SD values) of the stability constants are given in parentheses in Table 1. The stability constants of M^{2+}–OH systems were included in the calculations and were taken from the literature [46]. The charges of the complexes have been omitted in the text, Table and Figures. It is to note that due to high basicity of the (CH_3)–NH^+–R group, the $pK_{(NH^+)}$ values cannot be determined by pH-metric titrations and therefore are ignored in the calculation process. Thus, HL^{3-} is assumed in calculations as the fully deprotonated form of the ligands. The equilibrium models and the corresponding stability constants giving the best fits of the pH-metric titration curves are presented in Table 1.

2.4. ESI-MS measurements

ESI-MS experiments for the Cu(II)–ligand systems were performed on a Bruker MicrOTOF-Q spectrometer (Bruker Daltonics, Bremen, Germany) equipped with an Apollo II electrospray ionization source with an ion funnel. The spectra were acquired in both positive and negative ion mode. The experiments were performed for the solutions with 1:1.5 and 1:2 metal-to-ligand molar ratios. The pH of starting aqueous solutions was at *ca.* 6.5 and 10 by an addition of the desired amount of KOH. Routinely the 50:50 (v/v) MeOH/H_2O samples were measured. However, the variations of the analyte composition down to 5% of MeOH did not change the species composition. Samples were infused into the ESI source at a flow rate of 3 μl/min. Before each run, the instrument was calibrated externally with the Tunemix TM mixture (BrukerDaltonik, Germany) in quadratic regression mode. The instrumental parameters were as follows: the scan range *m*/*z* 200–2000, end plate offset −500 V, dry gas nitrogen (4 L/min), temperature 473 K, capillary voltage 4500 V, ion energy 5 eV. Data analysis was carried out with the use of Bruker Daltonics Data Analysis software v. 3.4.

2.5. EPR measurements

The EPR spectra were measured using a Bruker Elexsys E500 spectrometer equipped with an NMR teslameter (ER 036TM) and frequency counter (E 41 FC) at X-band frequency at 77 K and at room temperature. The simulations of experimental spectra were performed to reach the EPR parameters using computer program WINEPR SIMFONIA, version 1.26. Due to the strong absorption of microwave energy by water very narrow sample tubes were used for liquid solution measurements. The samples for EPR measurements were prepared in water/ethylene glycol (4:1 v/v) solution to ensure good glass formation with a copper(II) concentration of 4×10^{-3} mol dm^{-3} in all samples. The copper(II) ion to ligands molar ratios were the same as those used in the UV–Vis measurements. The solutions were usually measured at the same pH range as in the potentiometric studies. The pH of solutions was measured using Elmetron, CPC401 pH-meter with a combined pH electrode.

2.6. NMR measurements

Samples for ^1H and $^{31}P\{^1H\}$ NMR titration studies were prepared in D_2O with a ligand concentration of 1×10^{-2} M. Titrations were performed over a pH range *ca.* 2–13. The pH was measured using a Radiometer pH M83 instrument equipped with a Mettler Toledo INLAB 422 combined electrode and is given as meter readings without correction for pD.

2.7. UV–Vis measurements

Jasco V-570 spectrophotometer was used to record the electronic absorption spectra in the range of 300–900 nm at room temperature. The path length was 3 cm. The measurements were carried out in aqueous solution, at different pH values (0.5 pH unit step) between 2.0 and 12, at all metal to ligand molar ratios studied, and at 4×10^{-3} mol dm^{-3} copper(II) concentration. All the solutions were freshly prepared using bi-distilled water. The metal ion to ligand ratios for the copper(II):ligand system were 1:2 and 1:3.

3. Results and discussion

3.1. Synthesis of compounds *3a–g*

Target *N*-methyl alkylaminomethane-1,1-diphosphonic acids **3a–g** were prepared in two different ways. The first, one-pot, two step procedure is based on the reaction of secondary amines with dialkylphosphonates and trialkylorthoformates at elevated temperature [26,47]. Thus, the corresponding *N*-methyl alkylamines **1a** and **1b** were heated at 150–160 °C with diethylphosphonate and triethyl orthoformate until the evolution of the ethanol stopped. The crude tetraethyl esters **2a** and **2b** were used in the next step without further purification. Thus, the crude reaction mixture obtained at first step was hydrolyzed by refluxing with 6 M hydrochloric acid to give desired **3a** and **3b** with 36% and 52% yield, respectively (Scheme 2). Compound **3a** is new, and **3b** has been described in the literature. Maier synthesized **3b** by the reaction of *N*-benzyl-*N*-methylformamide with triethylphosphite in the presence of phosgene and subsequent acid hydrolysis [27]. Compound **3b** was also described by Ling et al., but without any experimental details and spectroscopic data [28].

Compounds **3c–g** were synthesized by the reductive methylation of mono *N*-substituted alkylaminomethane-1,1-diphosphonic acids **3c′–g′** *via* the Eschweiler–Clarke reaction [48–50]. Thus, refluxing of **3c′–g′** with formaldehyde and an excess of formic acid by 24 h yielded the final *N*-methyl alkylaminomethane-1,

Table 1
Protonation constants ($\log\beta_n^H$), cumulative formation constants ($\log\beta_{pqr}$ values) for the complexes formed with Cu(II)–L system, at T = 298 K, I = 0.2 mol dm^{-3} (KCl).

Compound		**3d**	**3a**	**3e**	**3f**	**3b**	**3c**	**3g**
Species	[a]p q r	R = *sec*-butyl	R = *n*-pentyl	R = 1-methylhexyl	R = cycloheptyl	R = Ph–CH2	R = Ph–$(CH_2)_2$	R = Ph–$(CH_2)_3$
H(HL)	0 1 1	8.68(1)	8.57(1)	8.72(2)	8.72(1)	8.51(1)	8.48(1)	8.53(1)
H_2 (HL)	0 1 2	13.39(1)	13.47(1)	13.51(2)	13.48(1)	13.35(2)	13.33(1)	13.41(1)
H_3(HL)	0 1 3		15.25	15.32(10)	15.55(12)			
$pK_{(PO_3H^-)}$		8.68	8.57	8.72	8.72	8.51	8.48	8.53
$pK_{(PO_3H^-)}$		4.71	4.90	4.79	4.76	4.84	4.85	4.88
$pK_{(PO_3H_2)}$			1.78	1.81	2.07			
[Cu(H_2L)]	1 1 1	13.10(3)	13.02(2)	13.28(2)	12.88(3)	13.01(4)	12.93(2)	12.94(2)
[$Cu_2(H_2L)_3$]	2 3 3	41.98(8)	41.74(5)	42.04(7)	41.78(5)	41.37(15)	41.32(5)	41.71(5)
[$Cu_2(H_2L)_2$(HL)]	2 3 2	37.92(4)	37.38(5)	37.97(5)	37.54(3)	37.46(5)	37.28(2)	37.46(3)
[$Cu_2(H_2L)$($HL)_2$]	2 3 1	33.22(6)	32.49(5)	33.06(7)	32.62(5)	32.47(8)	32.42(3)	32.62(4)
[Cu(HL)]	1 1 0	**8.41(6)**	**8.52(4)**	**8.85(3)**	**8.15(5)**	**8.28(8)**	**8.32(3)**	**8.33(4)**
[$Cu(HL)_2$]	1 2 0	13.82(5)	13.41(3)	13.82(4)	13.55(4)	13.38(7)	13.41(3)	13.42(4)
[CuL]	1 1 −1	−0.68(8)	−0.49(5)	−0.48(8)	−1.06(7)	−0.75(10)	−0.51(4)	−0.60(6)
[Cu(HL)L]	1 2 −1	3.83(7)	3.58(5)	3.66(5)	3.43(5)	3.75(10)	3.66(4)	3.48(5)
[CuL_2]	1 2 −2	−6.95(7)	−7.22(5)	−6.78(5)		−6.05(9)	−6.80(4)	−7.40(5)
$\log K_{[Cu(HL)_2]}$		5.41	4.89	4.97	5.40	5.10	5.09	5.09
$\log K_{[CuH_2L]} - pK_{PO_3H^-}$		8.39	8.12	8.49	8.12	8.17	8.08	8.06
$pK_{[Cu(H_2L)]}$		4.69	4.50	4.43	4.73	4.73	4.61	4.61
$pK_{[Cu(HL)_2]}$		9.99	9.83	10.16	10.12	9.63	9.75	9.94
$pK_{[Cu(HL)L]}$		10.78	10.80	10.44		9.80	10.46	10.88
$\log(K_{[Cu(HL)]}/K_{[Cu(HL)_2]})$		3.00	3.63	3.88	2.75	3.18	3.23	
χ^2		8.72	2.19	8.74	6.03	26.74	4.74	11.78
σ		7.86	4.72	4.93	7.39	8.39	4.03	5.82

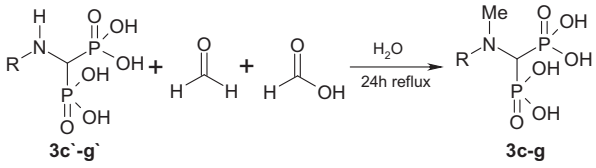
[a] The constants are calculated for the equilibrium: pM + qHL + rH = $M_pL_qH_{r+q}$.

1-diphosphonic acids **3c**–**g** in 80–97% (Scheme 3). Among compounds **3c**–**g**, only **3c** (*N*-methyl *Incadronate*) is known in the literature, prepared by Takeuchi et al. [26] by Eschweiler–Clarke methylation of tetraethyl ester of *Incadronate* and subsequent hydrolysis of obtained *N*-methylated tetraethyl ester. Our results have shown that direct Eschweiler–Clarke methylation of *Incadronate* gave better results.

Starting mono *N*-substituted alkylaminomethane-1,1-diphosphonic acids **3c′**–**f′** were prepared from appropriate primary amines using similar procedure as for **3a** and **3b**. 3-Phenylpropylaminomethane-1,1-diphosphonic acid (**3g′**) was obtained in the reaction of 3-phenylpropylisocyanide with two equivalents of triethylphosphite in the presence of stoichiometric amount of hydrochloride, followed by hydrolysis with 6 M hydrochloric acid [44] with 85% yield (Scheme 4).

3.2. Acid–base properties of *3a–g*

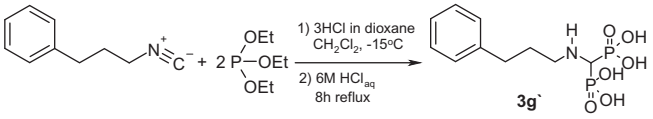
The compounds of interest (Schemes 1–4) contain five dissociable protons in the fully protonated form, $[H_5L]^+$. The first deprotonation step in the phosphonic (PO_3H_2) groups takes place in very acidic pH (*ca.* ~1.5–2) and therefore usually lies out of measurable pH range (2–11.5). In our case, the $pK_{(PO_3H_2)}$ values are determined only for **3a**, **3e** and **3f**. The subsequent deprotonation steps correspond to release of protons from phosphonate (PO_3H^-) groups (see Table 1). The values of deprotonation constants for these processes lie in the range of 4.71–4.90, 8.48–8.72 log units and correspond well with previously reported for other aminomethane-1,1-diphosphonic acids [20]. The last proton dissociates from the nitrogen atom of (CH_3)–NH^+–R group in strongly alkaline pH ($pK_{(NH^+)} > 13$), therefore the deprotonation constant for this process cannot be determined by pH-potentiometric titrations under experimental conditions [51]. The basicity of this group is higher as compared to NRH_2^+ group of previously studied compounds (Scheme 1a) and may be attributed to the inductive effect of electron attracting methyl substituent placed on the amino nitrogen atom. A clear evidence for very high basicity of (CH_3)–NH^+–R group is provided by ^{31}P and ^{1}H NMR titration curves of representative **3a** and **3f**, which in contrast to previously studied *N*-*n*-pentylaminomethane-1,1-diphosphonic acid [20] do not show any changes in chemical shifts versus pH in alkaline conditions (Fig. 1).



Scheme 3. Synthesis of the *N*-methyl alkylaminomethane-1,1-diphosphonic acids **3c**–**g** *via* Eschweiler–Clarke methylation.

3.3. Cu(II) complexes of *3a–g* in solution

ESI-MS experiments have been performed with the aim to establish stoichiometries of the complexes formed in Cu(II) solutions with ligands **3a**–**g**. This technique is not able to define the number of ionizable protons in the species, however, the determination of their molecular weight, charge, and isotopic distribution patterns offers a powerful tool for the elucidation of stoichiometries of metal complexes pre-formed in solution. Indeed, series of adducts with same M(II):ligand molar ratio and variable number of alkali cations seem to be a good indication of reliability of stoichiometric recognition [52,53]. The ESI-MS spectra of all studied systems exhibit peaks corresponding mostly to single positive and single negative molecular ions, which can easily be attributed to the 1:1, 1:2 and 2:3 M(II):L complexes. All peak assignments are



Scheme 4. Synthesis of 3-phenylpropylaminomethane-1,1-diphosphonic acid **3g′**.

confirmed by comparison of calculated and experimental isotope distribution patterns, as presented in Fig. 2 for the representative Cu(II)–**3a** (Fig. 2a–c) and Cu(II)–**3f** (Fig. 2d) systems.

In view of the above consideration, the stability constants of the complexes in the studied systems have been calculated based on the assumption that stoichiometries of the most chemically significant complexes are consistent with stoichiometries detected by ESI-MS method. The pH-potentiometric calculations have been performed with the use of approach, which neglects proton-dissociation from the highly basic (CH_3)–NH^+–R group and considers HL^{3-} ligand form as L^{3-} in the formation equilibrium $pM + qHL + rH = M_pL_qH_{r+q}$. This is acceptable when reliable $pK_{(NH^+)}$ values cannot be determined [54] and was previously used by us for evaluation of the stability constants of Zn(II), Mg(II) and Ca(II) complexes formed by bisphosphonates containing piperidyl-, morpholinyl- and thiomorpholinyl- side chains [21,51].

In general, titrations of respective ligand in the presence of various equivalents of Cu(II) have been analyzed in batch calculations, in which all titration curves are fitted at the same time with one model. The equilibrium models and corresponding stability constants giving the best fits of experimental and calculated pH-metric titration curves are collected in Table 1.

The pH-metric titrations indicate that the extent of complex formation in the copper(II) systems with ligands **3a**–**g** is quite similar. Each sample could be titrated without precipitation up to pH 10.5. The species distribution curves derived from the potentiometric calculations carried out in the pH range 2–10.5 do not differ considerably. Therefore, the Cu(II)–**3a** metal-to-ligand 1:2 molar ratio system was chosen to be representative for species distribution and variations of visible absorption maximum wavelengths as a function of pH (Fig. 3).

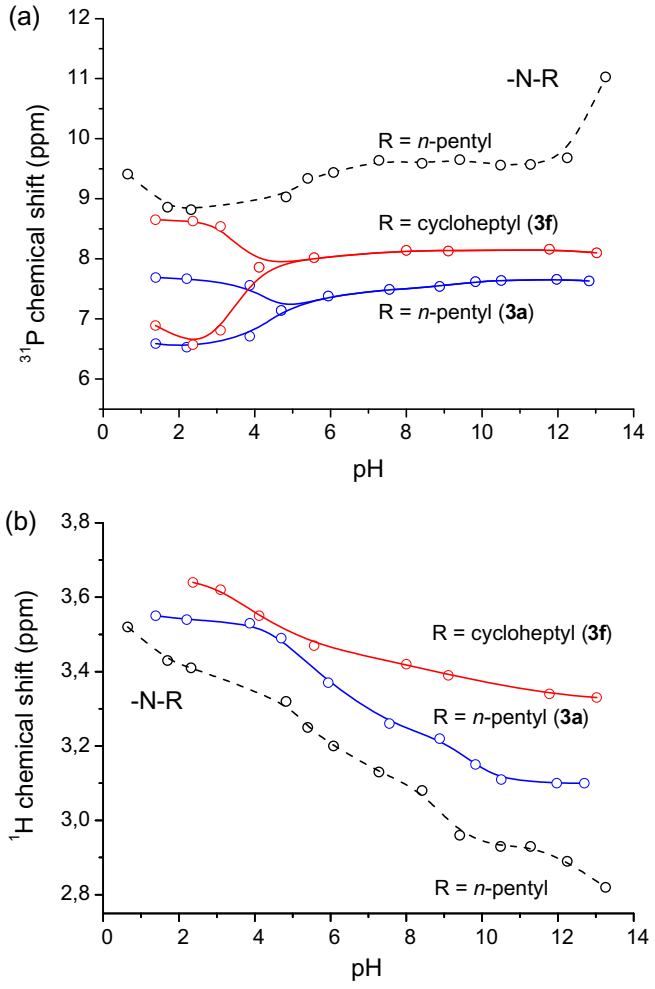


Fig. 1. (a) ^{31}P NMR and (b) ^{1}H NMR (CH proton) titration curves vs. pH for the compounds **3a**, **3f** and previously studied *N*-*n*-pentylaminomethane-1,1-diphosphonic acid [20]. Please note chemical non-equivalence of PO_3H_2/PO_3H^- groups of **3a** and **3f** reflected in their ^{31}P NMR spectra performed in acidic conditions.

The speciation calculated for the Cu(II)–**3a** 1:2 molar ratio system indicates that the process of complexation begins below pH 2 where $[Cu(H_2L)]$ and $[Cu_2(H_2L)_3]$ complexes start to form. These complexes are the only species present in equilibrium up to pH ~3 (Fig. 3). Spectral parameters calculated from the EPR spectra performed at pH ~2–3.3 ($g_{\|}$ = 2.418–2.425, $A_{\|}$ = 132−134 × 10^{-4} cm^{-1} and $g_{\perp}$=2.081–2.083) differ only slightly from those calculated for Cu(II) aqua ion ($g_{\|}$ = 2.415, $A_{\|}$ = 135 × 10^{-4} cm^{-1} and $g_{\perp}$ = 2.084 [22]. This indicates only minor changes in the donor set around copper(II) ion. It was reported that the displacement of water molecules around copper(II) ions by PO_3H^- or PO_3^{2-} donors does not greatly affect the EPR parameters [55]. Similarly, the energy of *d*–*d* transition at pH ~3 and λ_{max} ~805–798 nm with ε ~22–25 is typical for copper(II) complexes with pure oxygen surrounding. The $pK_{[Cu(H_2L)]}$ values for ligands **3a**–**g** (Table 1, row 5 from bottom), characteristic for the deprotonation step for the $[Cu(H_2L)]$ complexes, are close to the corresponding deprotonation constants of 'free' ligands (Table 1). Thus, they can be assigned to deprotonation of phosphonate PO_3H^- group. In [Cu(HL)] the ligand coordinates copper(II) with simple {O, O}-type bidentate manner using one oxygen atom of each suitably positioned phosphonate (PO_3^{2-}) groups to form the six-membered chelate ring (Scheme 5). Additionally, taking into account the difference in acidity of the coordinating phosphonate groups, the equilibrium constant $\log K_{[Cu(H_2L)]} - pK_{PO_3H^-}$, is roughly of the same order of magnitude as stability constants, $\log K_{[Cu(HL)]}$ (Table 1). This fact suggests the same {O, O} bidentate binding mode of the ligands in [Cu(HL)] and $[Cu(H_2L)]$ species (Scheme 5). The preference for such coordination is well documented in the literature [22,55,56,57].

A common feature of the Cu(II) systems with **3a**–**g** is the formation of oligonuclear species (most likely dinuclear complexes) besides the mononuclear ones (Table 1, Figs. 2 and 3). So, besides the $[Cu(H_2L)]$ complex, dinuclear $[Cu_2(H_2L)_3]$ species occur above pH 2.5 (maximum above 60% at pH ~3.5). The $[Cu_2(H_2L)_2(HL)]$ and $[Cu_2(H_2L)(HL)_2]$ species occur above pH 4.5 (Scheme 6); the latter one existing with a major contribution in the range of pH 4.5.0–7.5 (maximum above 80% at pH 6) and exists in an equilibrium with a another major contribution of $[Cu(HL)_2]$ (maximum above 85%, pH ~9) in the range of pH 6.5–7.5.

The EPR experiments clearly support the above indication. A pronounced formation of oligonuclear species demonstrated on the speciation diagrams (Fig. 3 as an example) is always accompanied by broadening of EPR signals in the acidic solution until pH 6.7 (Fig. 4, pH = 4.1 and 5.4). In addition, the obtained energy of *d*–*d* transition at pH range 4–6.5 (760–750 nm with ε ~38–42 mol^{-1} cm^{-1} dm^3, Fig. 3) agrees well with the energy reported for oligonuclear species formed by aminomethane-1,1-diphosphonic acids that contain oxygen donor atoms in coordination sphere of Cu(II) [57,22]. The ability of *gem*-phosphonate groups to coordinate metal ions in solutions through more than one oxygen atom each is well documented [19] and is also commonly observed in the solid state [58].

In all the studied systems the $[Cu(HL)_2]$ species are formed in basic solution despite their very high electric charge and considerable steric hindrance caused by two coordinated ligands. Interestingly, a distinct change of EPR spectral features is noticed as the pH of solution increases above pH 7 where mononuclear complexes are predicted (Fig. 4). The EPR spectra performed at pH about 6.5–9.5 reveal distinctly lower values of *g* parameter ($g_{\|}$ ~2.371–2.386 and $g_{\perp}$ ~2.065–2.071) and higher values of $A_{\|}$ parameter

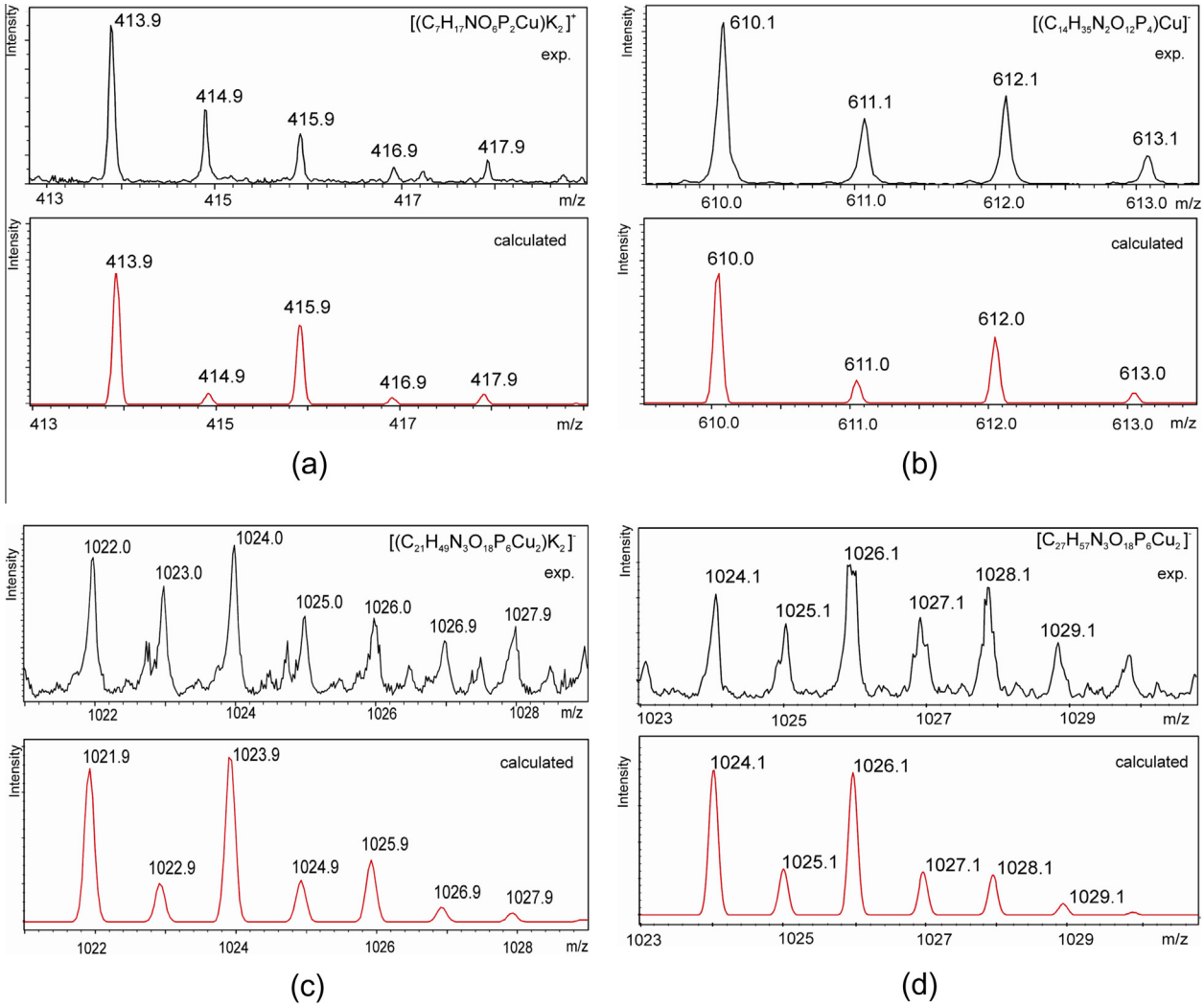


Fig. 2. Experimental and simulated isotope distribution patterns demonstrating the most significant Cu(II)–ligand stoichiometries detected in the ESI-MS spectra of representative Cu(II)–**3a** (a–c) and Cu(II)–**3f** (d) systems.

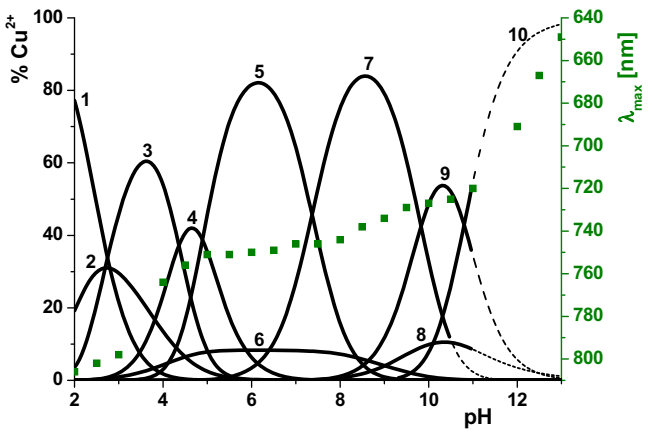
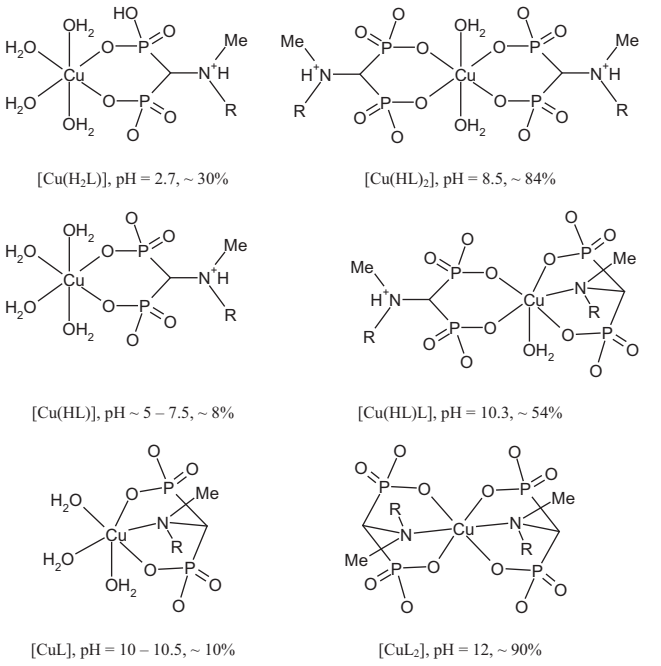


Fig. 3. Species distribution curves as a function of pH for conditions used in EPR and UV–Vis spectroscopy (solid line) and variations of visible absorption maximum wavelength (square) for the copper(II)–**3a** system at 1:2 molar ratio, $c_{Cu(II)} = 4 \times 10^{-3}$ mol dm^{-3}. 1 – Cu^{2+}; 2 – $[Cu(H_2L)]$; 3 – $[Cu_2(H_2L)_3]$; 4 – $[Cu_2(H_2L)_2(HL)]$; 5 – $[Cu_2(H_2L)(HL)_2]$; 6 – $[Cu(HL)]$; 7 – $[Cu(HL)_2]$; 8 – $[CuL]$; 9 – $[Cu(HL)L]$; 10 – $[CuL_2]$. The range simulated for conditions used in UV–Vis studies (dot line).

$(144–151 \times 10^{-4}$ cm$^{-1})$ as compared to those for $[Cu(H_2L)]$ species (see above). Furthermore, the energy of *d*–*d* transition for the $[Cu(HL)_2]$ species equal to 744 nm with $\varepsilon \sim 37$ mol^{-1} cm^{-1} dm^3 is higher in relation to the *d*–*d* absorption band of $[Cu(H_2L)]$ (Fig. 2). These spectroscopic results strongly support the involvement of second ligand in the Cu(II) coordination sphere. For this species the simple bidentate {O, O}-coordination through phosphonate PO_3^{2-} groups of both ligand molecules, resulting in the formation of two six-membered chelate rings, is the most probable (Scheme 5). Such type of binding mode with four phosphonate groups in the equatorial positions around metal center was reported for bis-complexes formed by various bisphosphonates [59].

A lot of data for stepwise stability constant ratios, $\log(K_{[Cu(HL)]}/K_{[Cu(HL)_2]})$, can be found in the literature [60]. Using the above expression for **3a**–**g**, we obtained the values given in Table 1 (row 3 from bottom). Their comparison shows that binding of second ligand is not so strongly hindered as for mono *N*-substituted aminomethane-1,1-diphosphonic acids (Scheme 1, left) [20]. For the systems with **3a**, **3d** and **3e** the values of $\log(K_{[Cu(HL)]}/K_{[Cu(HL)_2]})$ are evidently higher than for the systems with **3b**, **3c** and **3g**, which is likely due to the presence of phenyl ring in their side chains. The smallest value is obtained for the system with **3f** bearing bulky cycloheptyl ring (Table 1).

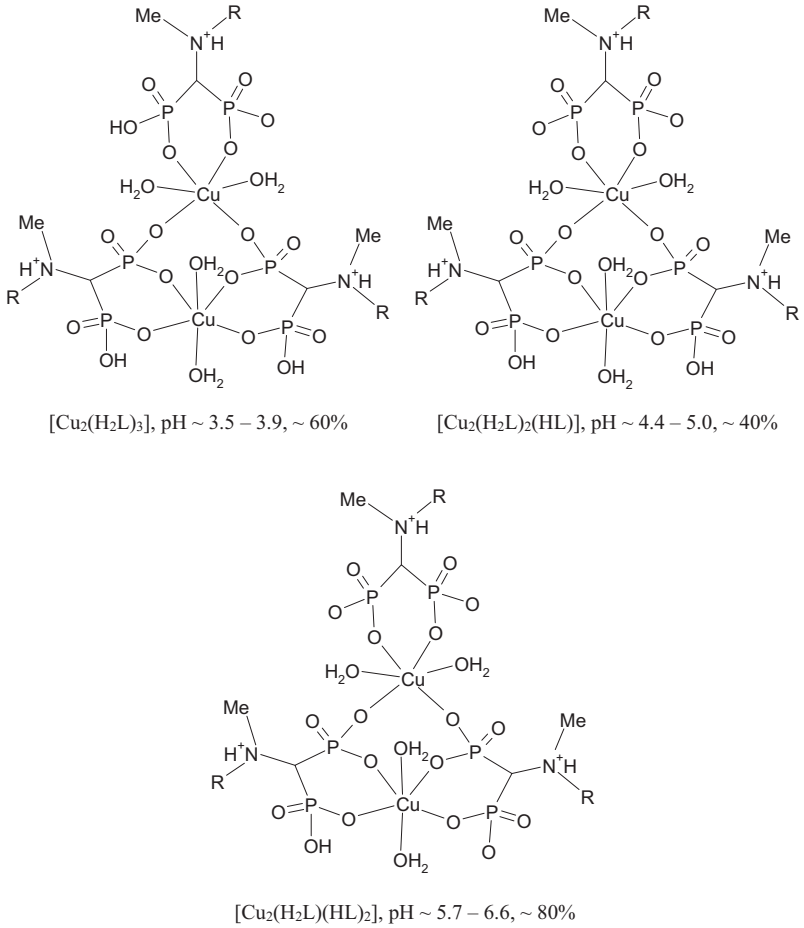


Scheme 5. Schematic representations showing the possible structures of the mononuclear complex species formed in the water solution of Cu(II)–L systems and pH range of their major concentration.

As shown on the species distribution diagram of the representative Cu(II)–**3a** system (Fig. 3), the [Cu(HL)L] complex starts to form above pH 8. Its formation is accompanied by detectable changes in electronic absorption spectra. The pH dependence of λ_{max} in the visible spectra collected for the Cu(II)–**3a** system shows a ~20 nm blue shift (the value of λ_{max} decreases from ~744 at pH ca. 8.0 to ~725–729 at pH ca. 10–10.5), which parallels the formation of the [Cu(HL)L] from $[Cu(HL)_2]$ species.

Above pH 9.5 consecutive species starts to form. This is completely deprotonated complex species $[CuL_2]$. Its formation is followed by the explicit changes in the EPR and electronic absorption spectra (Fig. 4). The EPR spectrum recorded at pH ~12 corresponding to $[CuL_2]$ complex with $g_{\|} = 2.239$, $A_{\|}$ ~201–203 $\times 10^{-4}$ cm^{-1} and $g_{\perp}$ ~2.052–2.053 parameters is apparently different from those recorded for the solutions where $[Cu(HL)_2]$ has been found to be predominant (Fig. 4). Moreover, the electronic absorption spectra collected for these systems in basic solutions show a spectacular blue shift (the value of λ_{max} decreases from ~725 at pH ca. 10.0 to ~670–650 above pH ca. 12–13), which parallels the formation of the $[CuL_2]$ species. Both EPR and visible spectral parameters are consistent with those reported for phosphonic species with the nitrogen donor(s) complexed to Cu(II) [61–64]. This allows anticipation that the coordination in these complexes is realized by both oxygen and nitrogen donor atoms as a result of Cu(II)-promoted nitrogen deprotonation (Scheme 5).

A comparison of relative stability constants $\log K_{[Cu(HL)]} - (pK_{(PO_3H^-)} + pK_{(PO_3H^-)})$ for **3a**, **3d** and **3e** (−4.95, −4.98, −4.66, respectively), **3b**, **3c** and **3g** (−5.07, −5.01, −5.08, respectively)



Scheme 6. Schematic representations showing the possible structures of the dinuclear complex species formed in the in the water solution of Cu(II)–L systems and pH range of their maximum concentration.

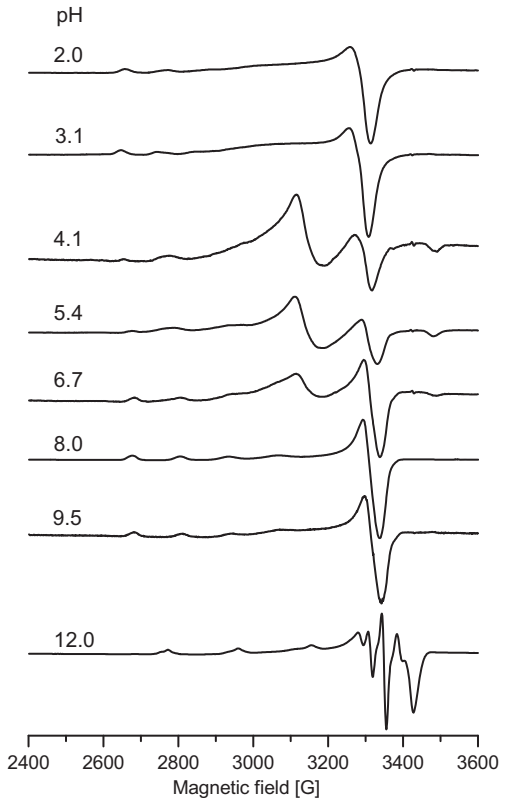


Fig. 4. The EPR spectra due to an aqueous solution containing Cu(II) ions and ligand **3a** depending on pH at 77 K for 1:3 metal-to-ligand molar ratios; $c_{Cu(II)} = 4 \times 10^{-3}$ M.

and **3f** (−5.33) reveals that despite similar basicities of coordinating donor groups, stabilities of [Cu(HL)] complexes differ and are strongly dependent on the nature of R substituent attached to the N atom (Schemes 1–4). Compounds **3a**, **3d** and **3e** with flexible alkyl and branched alkyl substituents on the N atom form the most stable complexes. Lower stability of [Cu(HL)] complexes of **3b**, **3c** and **3g** as compared to **3a**, **3d** and **3e** is probably caused by the presence of phenyl ring in R substituents. The stability of the least stable [Cu(HL)] complex of **3f** is *ca.* 0.4–1 log units lower compared to stability of **3a**–**e** and **3b**–**g**. This can be primarily attributed to the steric effect imposed by cycloheptyl ring attached directly to the N atom.

4. Conclusions

We described a synthesis of seven *N*-methyl alkylaminomethane-1,1-diphosphonic acids containing linear or branched alkyl, cycloheptyl or phenylalkyl R substituent on the nitrogen atom, isolated with average to good yields, and high purity, as well as their complex-formation abilities towards copper(II) in solution studied by means of pH-potentiometry, ESI-MS, EPR and NIR–Vis–UV methods.

Compounds **3a**–**g** demonstrate higher overall basicities and are better soluble compared to related mono *N*-substituted compounds. Evaluation of potentiometric data has revealed that metal complex speciation in the Cu(II)–ligand systems with **3a**–**g** is very similar. Overall, mostly protonated complexes with simple bidentate {O, O}-coordination through phosphonate groups have been observed over a broad range of pH. The [Cu(HL)L] and [CuL_2] complexes with bisphosphonate coordination realized by both oxygen and nitrogen donor atoms have been detected in strongly alkaline solutions. On the other hand, remarkable feature of **3a**–**g**, confirmed by ESI-MS and EPR experiments, is the formation of dinuclear [$Cu_2H_xL_3$] ($x = 4, 5, 6$) species besides of mononuclear [CuH_xL] and [CuH_xL_2] ones ($x = 0, 1, 2$). A comparison of stabilities of [Cu(HL)] complexes clearly indicates their strong dependence on electronic and steric effects imposed by R substituents attached to the nitrogen atom. Compounds with linear or branched alkyl on the nitrogen atom form the most stable complexes with Cu(II), the least stable are complexes of **3f** containing cycloheptyl ring attached directly to the nitrogen atom.

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