Coordination properties of cyclam (1,4,8,11-tetraazacyclotetradecane) endowed with two methylphosphonic acid pendant arms in the 1,4-positions†

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The title ligand, 1,4,8,11-tetraazacyclotetradecane-1,4-diyl-bis(methylphosphonic acid) (H_4te2p^{1,4}, H_4L), was prepared by an optimized synthetic approach and its complexing properties towards selected metal ions were studied by means of potentiometry. The ligand forms a very stable complex with copper(II) ($\log\beta$(CuL) = 27.21), with a high selectivity over binding of other metal ions (*e.g.* $\log\beta$(ZnL) = 20.16, $\log\beta$(NiL) = 21.92). The crystal structures of two intermediates in the ligand synthesis and two forms of the nickel(II) complex (obtained by crystallization at different pH) were determined. From acid solution, the crystals of *trans*-*O*,*O*-[Ni(H_3L)]Cl·H_2O were isolated. In such complex species, one phosphonate pendant arm is double- and the second arm is monoprotonated. The isolation of such species demonstrates a high kinetic inertness of the complex. The central metal ion is surrounded by four in-plane nitrogen atoms (in the ring configuration **III**) and two oxygen atoms of pendant moieties in the apical positions of octahedral coordination sphere. From neutral solution, the crystals of {*trans*-*O*,*O*-[Ni(H_2L)]}$_3$·5H_2O were isolated. The molecular structures of the complex units found in this structure are analogous to that found in *trans*-*O*,*O*-[Ni(H_3L)]Cl·H_2O.

Introduction

Most of the studies on macrocyclic ligands have been focused on derivatives bearing additional coordinating pendant groups which influence both thermodynamic stability and kinetics of the complexation/decomplexation. Among the macrocycles, 1,4,8,11-tetraazacyclotetradecane (cyclam, Chart 1) is one of the most studied azacycles.[1,2] Cyclams (fourteen-membered tetraamine macrocycles) show the ability to complex first-row transition metal cations, especially copper(II), and the complexes are often highly thermodynamically stable and kinetically inert.[3]

The investigations into the cyclam-derived ligands and their complexes is often motivated by their use in medicine.[3,4] It was found that cyclam-based anti-HIV agents are even more active *in vivo* in the form of complexes with several metal ions.[3,5] The macrocyclic ligands are used as chelators of the metal radioisotopes in targeted radiopharmaceuticals.[3,6] For the utilizations in nuclear medicine, macrocyclic ligands are generally preferred to open-chain ligands due to higher stability and usually higher inertness of their complexes. Among metal radioisotopes, the copper radionuclides (especially ^{64}Cu and ^{67}Cu) seem to be very promising. They undergo various decays, exhibit suitable properties for application in both scintigraphic imaging (single-photon imaging and positron emission tomography) as well as in targeted radiotherapy.[7] The ligands based on the cyclam skeleton offer an excellent binding environment for Cu(II) ion as the ion fits ideally in the macrocyclic cavity.[3,8] Chelators utilized for complexation of copper radionuclides are often based on 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid (H_4teta, Chart 1). However, such ligands have more donor atoms (8) than is required by copper(II) (5–6) and, therefore, several pendant arms remain non-coordinated in the copper(II) complexes.[9] It was shown, that such copper(II) complexes are not optimal for biomedical applications as they lose the metal ion *in vivo*.[10]

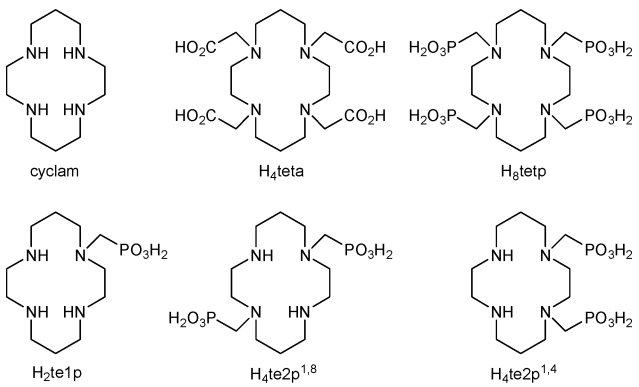

Chart 1 Structures of ligands mentioned in the text.

The investigations of macrocycles derivatized with four methylphosphonic acid (–CH_2–PO_3H_2)[11] or methylphosphinic acid (–CH_2–P(R)O_2H)[12] pendant arms (Chart 1) started several years ago. However, similarly to the H_4teta derivatives, the ligands have more coordinating atoms and the ligands should rather be considered as derivatives of *N*,*N*′,*N*″,*N*‴-tetramethylcyclam which forms generally kinetically less inert complexes than cyclam itself.[13] Therefore, copper(II) complexes of cyclam derivatives having a smaller number of pendant arms have

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† Electronic supplementary information (ESI) available: scheme of the ligand synthesis; molecular structures of the [Ni(H_2L)] complex units; NMR spectra of H_4L at different pH; distribution diagrams of the free ligand and the M(II)–H_4te2p^{1,4} systems (M = Ca, Pb, Ni, Zn, Cd). CCDC reference numbers 679184–679187. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/b803235a

been studied. The studies involved ligands with one carboxylic acid[14] or pyridine[15] pendant arm, or two acetate,[16,17] amine,[17] acetamide[17,18] or pyridine[19,20] coordinating pendant arms.

We have already prepared cyclam substituted with only one pendant arm[21] or with two methylphosphonic acid pendant arms in "*trans*" (1,8) positions (H_2te1p and H_4te2p[1,8], Chart 1).[22] Both ligands form extremely stable copper(II) complexes,[21,23] and the complexation reaction was found to be very selective for copper(II) over zinc(II).[24] We prepared the title ligand, 1,4,8,11-tetraazacyclotetradecane-1,4-diyl-bis(methylphosphonic acid) (H_4te2p[1,4], H_4L, Chart 1),[25] but a scaling up of the synthesis based on the original procedure was complicated by extensive chromatographic separations. So, we decided to develop another synthesis, based on previously reported oxalylbis(amide) protection.[26] Here, we report on the optimized ligand synthesis and the investigations into complexing properties of H_4te2p[1,4] towards selected divalent metal ions.

Experimental

Chemicals

Cyclam[27] and compounds **1**–**3**[26] were synthesised by published methods. Paraformaldehyde was filtered from aged aqueous solutions of formaldehyde (Lachema) and was dried in a desiccator over conc. H_2SO_4. All other chemicals were used as received from commercial sources. Metal ion stock solutions were prepared by dissolution of recrystallized $M(NO_3)_2 \cdot xH_2O$ in deionized water; the metal content was determined by titration with Na_2H_2edta solution. A standard nitric acid solution was prepared by passing an aq. potassium nitrate solution through a Dowex 50W-8 (H^+ form) column. A carbonate-free KOH (~0.2 M) solution was standardized against potassium hydrogen phthalate and the HNO_3 (~0.03 M) solution against the KOH solution.

Instrumental methods

NMR spectra were recorded on a Varian VNMRS300 spectrometer operating at 299.9 (^{1}H), 75.4 (^{13}C) and 121.4 (^{31}P) MHz. NMR spectra references: tetramethylsilane for $CDCl_3$ solutions (δ_H, δ_C = 0 ppm) and *t*-BuOH for D_2O solutions (δ_H = 1.25 ppm, δ_C = 32.0 ppm) as internal references and 85% D_3PO_4 in D_2O (δ_P = 0.0 ppm) as an external reference. Chemical shifts δ are given in ppm and coupling constants J are reported in Hz. Abbreviations of signal multiplicities: s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), m (multiplet) and br (broad). The ESI-MS spectra were acquired on a Bruker ESQUIRE 3000 spectrometer with ion-trap detection in both positive and negative modes. Thin layer chromatography (TLC) was performed on aluminium sheets Silica gel 60 F254 (Merck KGaA, Germany) using 2-propanol : conc. aq. NH_3 : water 7 : 3 : 3 mixture as mobile phase with ninhydrin spray or dipping of the sheets in 5% aq. solution of $CuSO_4$ detection. Elemental analyses were done in the Institute of Macromolecular Chemistry (Academy of Sciences of the Czech Republic, Prague).

Synthesis

Isolation of 1,5,8,12-tetraazabicyclo[10.2.2]hexadecane-13,14-dione, 1. Due to problematic isolation of **1** as a free base according to ref. 26, we isolated the compound as dihydrochloride salt as follows. The reaction mixture from the original synthesis[26] (5 mmol scale, 1.00 g of cyclam) was evaporated to dryness, and re-dissolved in 15 ml of ethanol. Concentrated aq. HCl (1 ml) was added dropwise and the white precipitate was collected by filtration and dried in a desiccator (1.36 g, 83%). Found: C, 43.9; H, 7.2; Cl, 22.0; N, 17.0%. Calc. for **1**·2HCl, $C_{12}H_{24}Cl_2N_4O_2$, M = 327.3: C, 44.0; H, 7.4; Cl, 21.7; N, 17.1%. Colourless needles of **1**·2HCl suitable for X-ray diffraction were prepared by vapour diffusion of ethanol into aq. solution of **1**·2HCl, acidified by a drop of a diluted aq. HCl.

Isolation of 1,4-dibenzyl-1,4,8,11-tetraazacyclotetradecane, 3. After hydrolysis of **2** in aq. NaOH and extraction of **3** into chloroform and evaporation,[26] the compound was isolated by crystallization from concentrated aq. HBr (75%, based on **1**·2HCl) as an off-white solid. Found: C, 37.7; H, 6.0; Br, 41.6; N, 7.2%. Calc. for **3**·4HBr·4H_2O, $C_{24}H_{48}Br_4N_4O_4$, M = 776.3: C, 37.1; H, 6.2; Br, 41.2; N, 7.2%. Colourless plates of **3**·4HBr·2H_2O were formed by slow cooling of a hot solution of **3** in diluted (1 : 1) aq. HBr.

Synthesis of 8,11-dibenzyl-1,4-bis(diethoxyphosphorylmethyl)-1,4,8,11-tetraazacyclotetradecane, 4. Compound **3**·4HBr·4H_2O (5.45 g, 7.0 mmol) was dissolved in water (100 ml) and NaOH (5 g) was added. Free base **3** was extracted by $CHCl_3$ (3 × 50 ml) and the extracts were combined and dried over Na_2SO_4. The mixture was filtered and the solvent was evaporated. The macrocycle was dissolved in triethyl phosphite (11.6 g, 74 mmol, 10 equiv.). Paraformaldehyde (1.10 g, 37 mmol, 5 equiv.) was added and the mixture was stirred at 75 °C for 4 d in a closed flask. The reaction mixture was poured onto a column of strong cation exchanger (Dowex 50, 100 ml, H^+ form). The non-cyclic compounds were eluted by EtOH : water (3 : 1, 500 ml) mixture. The product **4** was collected by EtOH : conc. aq. NH_3 (5 : 1, 250 ml) mixture. After evaporation, the product was isolated as a brownish oil. TLC (ninhydrin) brown spot, R_f = 0.9; δ_H($CDCl_3$) 1.31 (12 H, m, CH_3), 1.62 (4 H, p, $^3J_{HH}$ 6.8, $CH_2CH_2CH_2$), 2.59 (4 H, t, $^3J_{HH}$ 6.8, $NCH_2CH_2CH_2N$), 2.62 (4 H, s, NCH_2CH_2N), 2.70 (4 H, t, $^3J_{HH}$ 6.8, $NCH_2CH_2CH_2N$), 2.79 (4 H, s, NCH_2CH_2N), 2.95 (4 H, d, $^2J_{PH}$ 10.0, CH_2P), 3.48 (4 H, s, CH_2Ph), 4.12 (8 H, m, OCH_2) and 7.2–7.6 (10 H, m, arom.); δ_C($CDCl_3$) 17.0 (4 C, CH_3), 25.0 (2 C, $CH_2CH_2CH_2$), 51.0 (2 C, d, $^1J_{PC}$ 628, CH_2P), 51.2 (2 C, $PhCH_2NCH_2$), 52.4 (2 C, $PhCH_2NCH_2$), 53.2 (2 C, d, $^3J_{PC}$ 35.2, PCH_2NCH_2), 54.1 (2 C, d, $^3J_{PC}$ 35.2, PCH_2NCH_2), 60.3 (2 C, $PhCH_2$), 63.3 (4 C, d, $^2J_{PC}$ 148, OCH_2), 128.3 (2 C, arom.), 129.3 (4 C, arom.), 130.5 (4 C, arom.), 140.0 (2 C, arom. CCH_2); δ_P($CDCl_3$) 31.1 (br s).

Synthesis of 8,11-dibenzyl-1,4,8,11-tetraazacyclotetradecane-1,4-diyl-bis(methylphosphonic acid), 5. The compound **4** obtained above was dissolved in diluted aq. HCl (1 : 1, 70 ml) and the mixture was heated under reflux for 24 h. The volatiles were evaporated and the residue was dissolved in a small amount of water and poured onto a column of strong cation exchanger (Dowex 50, 100 ml, H^+ form). The column was washed by water till neutrality of the eluate and the product **5** was collected with 5% aq. HCl. Fractions containing the product were evaporated, re-dissolved in water and chromatographed on a column of weak cation exchanger (Amberlite CG50, 50 ml, H^+ form) with 3% aq. HCl as a mobile phase. Fractions containing pure product (TLC

check) were combined, and the product was crystallized from hot water with a few drops of conc. aq. HCl. The white hydrochloride salt was collected by filtration and dried in air (1.66 g, 33%, based on **3**·4HBr·4H_2O). Found: C, 43.3; H, 7.0; Cl, 15.2; N, 7.7; P, 8.6%. Calc. for **5**·3HCl·2H_2O, $C_{26}H_{49}Cl_3N_4O_8P_2$, M = 714.0: C, 43.7; H, 6.9; Cl, 14.9; N, 7.8; P, 8.7%; TLC (ninhydrin) purple spot, R_f = 0.6; δ_H(D_2O/NaOD, pD = 13.8) 1.68 (4 H, br, $CH_2CH_2CH_2$), 2.53 (8 H, br + s, $CH_2CH_2CH_2$ + NCH_2CH_2N), 2.64 (4 H, d, $^2J_{PH}$ 10.7, CH_2P), 2.75 (4 H, br, $CH_2CH_2CH_2$), 2.86 (4 H, s, NCH_2CH_2N), 3.49 (4 H, s, CH_2Ph), 7.16 (4 H, m, arom.) and 7.32 (6 H, m, arom.); δ_C(D_2O/NaOD, pD = 13.8) 21.2 (2 C, *C*C*C*), 45.4 (2 C, *C*CC), 49.8 (2 C, CC*C*), 52.7 (2 C, N*C*CN), 53.2 (2 C, NC*C*N), 57.0 (2 C, d, $^1J_{CP}$ 142, *C*P), 61.4 (2 C, *C*Ph), 129.9 (2 C, arom.), 130.8 (4 C, arom.), 132.1 (4 C, arom.), 139.4 (2 C, arom.); δ_P(D_2O/NaOD, pD = 13.8) 16.7 (t, $^2J_{PH}$ 11.8); *m*/*z* (ESI) +569.4 ([M + H]$^+$, $C_{26}H_{43}N_4O_6P_2^+$ requires 569.3), –567.3 ([M – H]$^-$, $C_{26}H_{41}N_4O_6P_2^-$ requires 567.3).

Attempt of synthesis of 1,4-bis(diethoxyphosphorylmethyl)-1,4,8,11-tetraazacyclotetradecane, 6. Compound **4** (0.32 g) was dissolved in EtOH (10 ml) in double-necked flask (50 ml) under argon. To the solution, a 10% Pd/C catalyst (0.20 g) was added. The flask was flushed with hydrogen, and the mixture was stirred under hydrogen atmosphere for 12 h. A new portion of catalyst (0.20 g) was added and the mixture was stirred for further 24 h. After that time, a sample of the mixture was filtered, evaporated to dryness and analyzed by NMR. According to ^{1}H NMR, complete debenzylation occurred, but three major peaks appeared in the ^{31}P NMR spectrum at 29.5 (15%), 31.3 (65%) and 31.8 (20%) ppm. Trial of de-esterification of the product mixture in dry HBr–AcOH solution (30% w/w) led to a more complicated mixture (5 peaks in ^{31}P NMR spectrum at 11.1 (15%), 11.6 (15%), 11.9 (40%), 12.9 (20%) and 14.0 (10%) ppm).

Synthesis of 1,4,8,11-tetraazacyclotetradecane-1,4-diyl-bis(methylphosphonic acid), H_4te2p^{1,4} (H_4L)

Compound **5**·3HCl·2H_2O (1.40 g, 2.1 mmol) was dissolved in a mixture of water (15 ml) and AcOH (10 ml) in a double-necked flask (50 ml) under argon. To the solution, a 10% Pd/C catalyst (0.15 g) was added. The flask was flushed with hydrogen and the mixture was stirred under a hydrogen atmosphere for 24 h. The catalyst was filtered off and the solution was evaporated to dryness. The residue was dissolved in a small amount of water and poured onto a column of strong cation exchanger (Dowex 50, 100 ml, H^+ form). The column was washed by water till neutrality of the eluate and the product H_4te2p^{1,4} was collected by 5% aq. NH_3. Fractions containing the product were evaporated to dryness. The residue was re-dissolved in water and chromatographed on a column of weak cation exchanger (Amberlite CG50, 50 ml, H^+ form) with water. The product was crystallized from conc. aq. solution by slow addition of acetone. The white solid H_4te2p^{1,4}·4H_2O was collected by filtration and dried in air (0.52 g, 54%). The characteristics were identical to those reported.[25]

Preparation of *trans*-*O*,*O*-[Ni(H_3L)]Cl·H_2O

Ligand (50 mg of H_4te2p^{1,4}·4H_2O, 0.11 mmol) was dissolved with an equimolar amount of $NiCl_2$·6H_2O (26 mg) in water (2 ml) and the solution was heated in a closed vial at 100 °C overnight. The complex formation was evident from colour change (green to violet) and MS (*m*/*z* (ESI) +445.2 ([M + H]$^+$, $C_{12}H_{29}N_4NiO_6P_2^+$ requires 445.1), –443.0 ([M – H]$^-$, $C_{12}H_{27}N_4NiO_6P_2^-$ requires 443.1)). The solution was concentrated to ~0.5 ml. Violet plates of *trans*-*O*,*O*-[Ni(H_3L)]Cl·H_2O were formed by a slow vapour diffusion of acetone and analyzed by X-ray crystallography.

Preparation of {*trans*-*O*,*O*-[Ni(H_2L)]}$_3$·5H_2O

Ligand (50 mg of H_4te2p^{1,4}·4H_2O, 0.11 mmol) was dissolved in water (2 ml) and mixed with excess of freshly prepared Ni(OH)$_2$ (prepared from 52 mg of $NiCl_2$·6H_2O by addition of diluted aq. NaOH and centrifugation). The solution was stirred overnight at room temperature and unreacted Ni(OH)$_2$ was filtered off. The complex formation was evident from the colour of the solution (violet) and MS (*m*/*z* (ESI) +445.2 ([M + H]$^+$, $C_{12}H_{29}N_4NiO_6P_2^+$ requires 445.1), –443.0 ([M – H]$^-$, $C_{12}H_{27}N_4NiO_6P_2^-$ requires 443.1)). The single crystals—violet-blue plates—of {*trans*-*O*,*O*-[Ni(H_2L)]}$_3$·5H_2O were formed by a slow vapour diffusion of acetone.

Crystallography

Selected crystals were mounted on a glass fibre in random orientation and cooled to 150(1) K. The diffraction data were collected employing a Nonius Kappa CCD diffractometer (Enraf-Nonius) using Mo-Kα (λ = 0.71073 Å) at 150(1) K (Cryostream Cooler, Oxford Cryosystem) and analyzed using the HKL DENZO program package.[28] The structures were solved by direct methods and refined by full-matrix least-squares techniques (SIR92 (ref. 29) and SHELXL97 (ref. 30)). The used scattering factors for neutral atoms were included in the SHELXL97 program.

In the structure of **1**·2HCl, all non-hydrogen atoms were refined anisotropically. The hydrogen atoms attached to carbon and nitrogen atoms were located in the electron density difference map; however, the hydrogen atoms attached to carbon atoms were fixed in theoretical positions using U_{eq}(H) = 1.2U_{eq}(C).

In the structure of **3**·4HBr·2H_2O, an independent unit is formed by one half of the amine molecule (the molecule lies on the crystallographic double-fold axis), two bromide anions and one solvate water molecule. The bromide anions occupy three positions (one with full and two with half crystallographic occupancies). Most of hydrogen atoms were located in the electron density difference map; however, due to the presence of a heavy atom, all hydrogen atoms were fixed in theoretical (C–H, N–H) or original (O–H) positions using U_{eq}(H) = 1.2U_{eq}(X). Due to a high absorption coefficient, absorption correction (Gaussian integration[31]) with scaling factors T_{min} = 0.2902 and T_{max} = 0.7003 was applied.

In the structure of *trans*-*O*,*O*-[Ni(H_3L)]Cl·H_2O, all non-hydrogen atoms were refined anisotropically. The hydrogen atoms were located in the electron density difference map; however, due to the presence of a heavy atom, all hydrogen atoms were fixed in theoretical (C–H, N–H) or original (O–H) positions using U_{eq}(H) = 1.2U_{eq}(X).

In the structure of {*trans*-*O*,*O*-[Ni(H_2L)]}$_3$·5H_2O, three complex molecules and five water solvate molecules form the structurally independent unit. All non-hydrogen atoms were refined anisotropically (except of those belonging to the disordered phosphonate)

and the hydrogen atoms attached to carbon and nitrogen atoms were fixed in theoretical positions using $U_{eq}(H) = 1.2U_{eq}(X)$. One phosphonate pendant group was best refined as disordered in two positions (with the common carbon and nickel-coordinated oxygen atoms). A low-quality of crystal data and a high number of refined parameters led to high uncertainties; therefore, some atoms have relatively high thermal parameters and, so the hydrogen atoms of water solvate molecules could not be located in the electron density map.

Crystal data. **1**·2HCl, $C_{12}H_{24}Cl_2N_4O_2$, $M = 327.25$, monoclinic, $a = 9.6533(3)$ Å, $b = 15.5059(4)$ Å, $c = 10.3121(2)$ Å, $\beta = 93.5297(16)°$, $U = 1540.62(7)$ Å^3, space group $P2_1/c$ (no. 14), $Z = 4$, 3533 reflections measured, 2869 unique, the final w$R_2 = 0.0798$ (all data). CCDC 679187.

3·4HBr·2H_2O, $C_{24}H_{44}Br_4N_4O_2$, $M = 740.27$, monoclinic, $a = 7.27000(10)$ Å, $b = 20.5765(4)$ Å, $c = 20.0208(4)$ Å, $\beta = 99.6839(13)°$, $U = 2952.26(9)$ Å^3, space group $C2/c$ (no. 15), $Z = 4$, 3408 reflections measured, 2854 unique, the final w$R_2 = 0.0659$ (all data). CCDC 679184.

trans-*O*,*O*-[Ni(H_3L)]Cl·H_2O, $C_{12}H_{31}ClN_4NiO_7P_2$, $M = 499.51$, orthorhombic, $a = 7.9420(2)$ Å, $b = 9.4016(3)$ Å, $c = 27.8565(8)$ Å, $U = 2079.98(10)$ Å^3, space group $P2_12_12_1$ (no. 19), $Z = 4$, 4739 reflections measured, 4236 unique, the final w$R_2 = 0.0872$ (all data). CCDC 679186.

{*trans*-*O*,*O*-[Ni(H_2L)]}$_3$·5H_2O, $C_{36}H_{94}N_{12}Ni_3O_{23}P_6$, $M = 1425.18$, hexagonal, $a = 15.2662(1)$ Å, $c = 43.2280(4)$ Å, $U = 8724.84(11)$ Å^3, space group $P6_1$ (no. 169), $Z = 6$, 13 307 reflections measured, 9407 unique, the final w$R_2 = 0.1673$ (all data). CCDC 679185.

Potentiometric titrations

Equilibria in systems of the ligand in free form (protonation study) and with Ca(II), Cu(II), Zn(II), Cd(II) and Pb(II) were established fast and, thus, they could be studied by conventional titrations. Titrations were carried out in a thermostatted vessel at 25.0 ± 0.1 °C, at constant ionic strength $I(KNO_3) = 0.1$ M, using a PHM 240 pH-meter, a 2 ml ABU 900 automatic piston burette and a GK 2401B combined electrode (all Radiometer). The concentration of the ligand was approximately 0.004 M. The ligand-to-metal ratio was 1 : 1 in all cases and the initial volume was about 5 ml. The measurements were taken with an addition of excess of HNO_3 to the mixture. The starting value of –log[H^+] was typically 1.65 (the Cu(II) system) or 1.8 (other systems). The mixtures were titrated with the solution of KOH till –log[H^+] of about 12.5. Titrations of each system were carried out at least four times. Each titration consisted of about 40 points (with approximately the same number of data points in both acid and alkaline regions). An inert atmosphere was ensured by a constant passage of argon saturated with water vapour.

The complexation reaction was too slow to be followed by standard titrations in the Ni(II) system. Thus, this system was studied by the "out-of-cell" method. Three titrations were done, each consisting of 30 points (*i.e.* from 30 solutions mixed separately in test tubes). Initial volume of the samples was about 1 ml. The tubes were left firmly closed for 1 h, then an appropriate amount of the KOH solution was added and the tubes were tightly closed. The potential was then determined with a freshly calibrated electrode after 3 weeks.

The water ion product was taken from literature (p$K_w = 13.78$).[32] The constants with their standard deviations were calculated with the OPIUM program package.[33] The program minimises the criterion of the generalised least-squares method using the calibration function

$$E = E_0 + S \times \log[H^+] + j_1 \times [H^+] + j_2 \times K_w/[H^+],$$

where the additive term E_0 contains the standard potentials of the electrodes used and the contributions of inert ions to the liquid-junction potential. The term S corresponds to the Nernstian slope and the $j_1 \times [H^+]$ and $j_2 \times [OH^-]$ terms are contributions of the H^+ and OH^- ions to the liquid-junction potential. They cause a deviation from a linear dependence between E and –log[H^+] only in strongly acidic or strongly alkaline solutions. The calibration parameters were determined from titration of the standard HNO_3 with the standard KOH solutions before and after every titration of the ligand (ligand/metal ion system) to give calibration–titration pairs used for calculations of the constants. The protonation constants β_h are defined as concentration constants $\beta_h = [H_hL]/([H]^h \times [L])$. The concentration stability constants β_{hlm} are defined by the equation $\beta_{hlm} = [H_hL_lM_m]/([H]^h \times [L]^l \times [M]^m)$. In the definitions, the charges of the species are omitted for clarity.

NMR titrations

The ^{31}P NMR titration experiments for determination of the highest protonation constants of the ligand (–log[H^+] 11.6–13.5) were carried out by the recommended method[34] under the conditions of potentiometric titrations (H_2O, 0.1 M KNO_3, 25.0 °C, 0.004 M ligand); however, with no control of ionic strength at the last points above –log[H^+] 13. A coaxial capillary with D_2O was used for a lock. The protonation constant was calculated with OPIUM from the dependence of δ_P on –log[H^+].

Results and discussion

Syntheses

Originally, H_4te2p[1,4] was prepared *via* thiophosphonylbis(amide) protection of 1,4-positions of the cyclam ring,[25] but scaling up the synthesis was complicated due to extensive chromatographic purifications. Thus, we proposed a new synthetic pathway, employing known oxalylbis(amide)- and bis(benzyl)-protected cyclams **1**, **2** and **3** (Scheme S1†).[26] However, in the original article, the authors isolated the oxalylcyclam **1** by simple crystallization which did not work in our hands. Therefore, we decided to isolate the compound **1** in the form of dihydrochloride which precipitated easily from ethanol solution of **1** on addition of conc. aq. HCl. The dibenzylcyclam **3** was isolated after crystallization from conc. aq. HBr acid as **3**·4HBr·4H_2O. Originally, we tried the crystallization from conc. aq. HCl, but the hydrochloride precipitated in an extremely fine form and, thus, was hardly filterable. The dibenzylcyclam **3** reacted with triethyl phosphite and paraformaldehyde forming protected intermediate **4** in a quantitative yield (according to ^{31}P NMR). After removal of excess formaldehyde and phosphite, the protecting benzyl groups as well as phosphonate ethyl ester groups were cleaved. First, we tried reductive debenzylation of the ester **4** in presence of Pd/C. This procedure led to a complicated mixture of products instead of pure

compound **6**. Further hydrolysis of ester moieties (HBr–AcOH) resulted in inseparable mixture of products. Therefore, the ester groups were hydrolyzed first to obtain acid **5** and benzyl groups were removed in the last step to obtain H_4te2p[1,4] in an overall moderate yield.

Crystal structures

Crystal structure of 1·2HCl. Compound **1**·2HCl crystallized in the form of colourless needles. The independent unit of the crystal structure is formed by one macrocyclic molecule and two chloride counter-ions. The six-membered cycle (Fig. 1) formed by oxalyl group bonded to macrocycle nitrogen atoms N1 and N4 is perpendicular to the plane of the rest of the macrocycle. The crystal structure is stabilized by intermolecular hydrogen bond network between protonated amino groups, chloride anions ($d_{N\cdots Cl}$ ~ 3.0–3.1 Å) and oxygen atoms belonging to the neighbouring molecule ($d_{N\cdots O\#}$ ~ 2.8–2.9 Å).

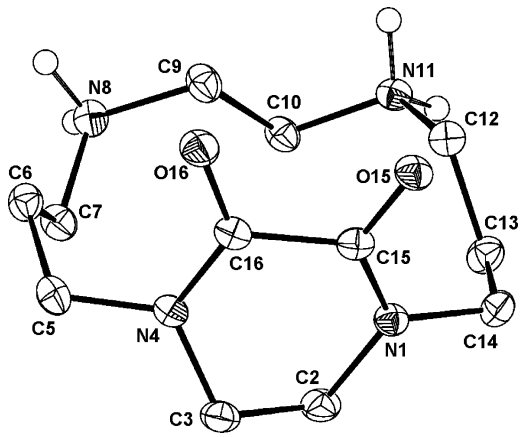

Fig. 1 Molecular structure of the $H_2\mathbf{1}^{2+}$ dication found in the crystal structure of **1**·2HCl. Hydrogen atoms on carbon atoms are omitted for the sake of clarity. Thermal ellipsoids show 50% probability level.

Crystal structure of 3·4HBr·2H$_2$O. In the structure of **3**·4HBr·2H_2O, all amino groups are protonated. The macrocycle is in the most common rectangular conformation (3,4,3,4)-A with all amino groups lying in the corners of the rectangle (Fig. 2).[2] The whole structure is stabilized by intermolecular hydrogen bonds

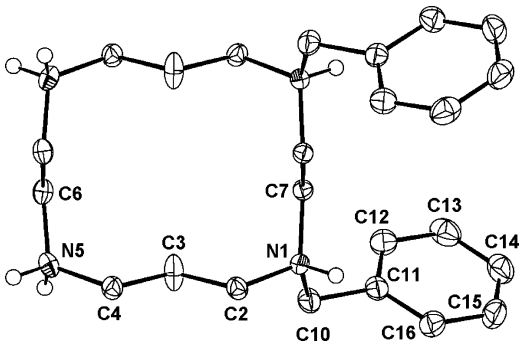

Fig. 2 Molecular structure of the $H_4\mathbf{3}^{4+}$ tetracation found in the crystal structure of **3**·4HBr·2H_2O. Hydrogen atoms on carbon atoms are omitted for the sake of clarity. Thermal ellipsoids show 50% probability level.

between protonated amino groups, bromide anions ($d_{N\cdots Br}$ ~ 3.2 Å) and oxygen atoms of solvate water molecule ($d_{N\cdots O}$ ~ 2.7 Å). There are also contacts between solvate water hydrogen atoms and the bromides ($d_{O\cdots Br}$ ~ 3.3–3.4 Å).

Crystal structure of *trans*-*O*,*O*-[Ni(H$_3$L)]Cl·H$_2$O. The independent unit is formed by a complex molecule, a chloride anion and a solvate water molecule. Coordination sphere of Ni(II) ion is octahedral, with four macrocycle nitrogen atoms in the equatorial plane and two apically coordinated oxygen atoms of the phosphonate pendant arms (Fig. 3). Coordination bond lengths have common values for these types, with slightly longer bonds (by ~0.06 Å) between the central ion and the tertiary amino groups compared to the secondary ones (Table 1). The macrocycle is in the most stable configuration **III**,[35] which is the most common configuration found for Ni(II) complexes with cyclam-like ligands.[36] The geometries around the phosphorus atoms are roughly tetrahedral. One phosphonate is monoprotonated, and the other is double-protonated on the non-coordinated oxygen atoms. The isolation of such a species points to a high inertness of the complex, as the second protonation step of the coordinated phosphonate group proceeds typically with pK_a ~ 1.[23,37,38] Similar over-protonated species were isolated also in the case of Ni(II) complexes of H_4te2p[1,8] (ref. 37). The orientation of phosphonate pendant arms above and below the macrocyclic plane is fixed by intramolecular hydrogen bonds between hydrogen atom of secondary amino group (hydrogen atoms attached to N8 and

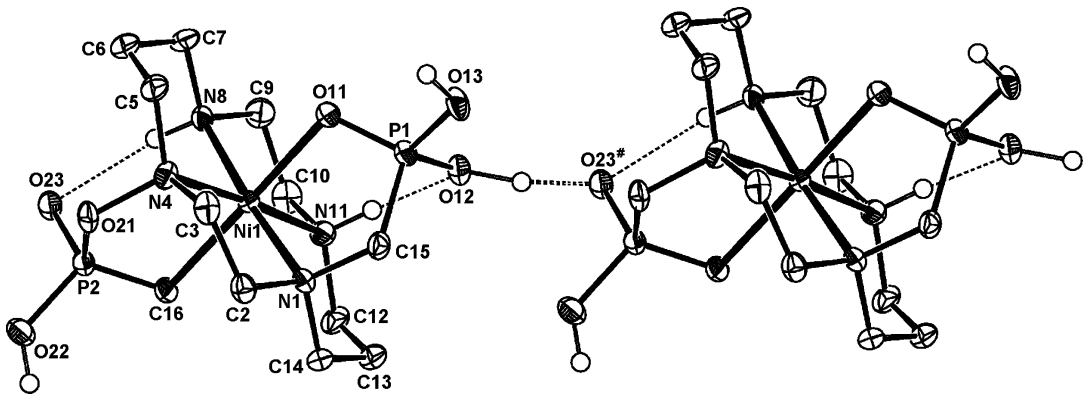

Fig. 3 Molecular structure of the complex units found in the crystal structure of *trans*-*O*,*O*-[Ni(H$_3$L)]Cl·H$_2$O, showing the hydrogen bond system (dashed). Hydrogen atoms attached to carbon atoms are omitted for the sake of clarity. Thermal ellipsoids show 50% probability level.

Table 1 Selected geometric parameters of the nickel(II) coordination spheres in the crystal structures of *trans-O,O*-[Ni(H_3L)]Cl·H_2O and {*trans-O,O*-[Ni(H_2L)]}$_3$·5H_2O

		{*trans-O,O*-[Ni(H_2L)]}$_3$·5H_2O		
	trans-O,O-[Ni(H_3L)]Cl·H_2O	Molecule A	Molecule B	Molecule C
Distances/Å				
Ni1–N1	2.130(3)	2.14(1)	2.13(1)	2.16(1)
Ni1–N4	2.136(3)	2.09(1)	2.10(1)	2.13(1)
Ni1–N8	2.076(3)	2.06(1)	2.06(1)	2.07(1)
Ni1–N11	2.076(3)	2.08(1)	2.07(1)	2.08(1)
Ni1–O11	2.106(2)	2.14(1)	2.14(1)	2.09(1)
Ni1–O21	2.119(2)	2.09(1)	2.09(1)	2.10(1)
Angles/°				
N1–Ni1–N4	86.5(1)	86.6(4)	88.5(3)	86.1(5)
N1–Ni1–N8	175.4(1)	176.7(5)	173.1(5)	174.7(4)
N1–Ni1–N11	94.1(1)	95.7(4)	93.8(5)	94.6(5)
N1–Ni1–O11	86.1(1)	86.7(5)	85.3(5)	94.6(5)
N1–Ni1–O21	91.8(1)	91.3(5)	93.1(5)	93.1(4)
N4–Ni1–N8	94.5(1)	91.9(5)	91.6(5)	93.3(5)
N4–Ni1–N11	175.5(1)	172.9(6)	174.9(5)	176.8(5)
N4–Ni1–O11	94.1(1)	95.1(6)	93.3(5)	90.1(4)
N4–Ni1–O21	85.7(1)	84.7(6)	86.2(5)	86.3(4)
N8–Ni1–N11	85.3(1)	86.1(3)	86.8(3)	86.3(5)
N8–Ni1–O11	89.3(1)	90.5(5)	87.9(4)	86.3(5)
N8–Ni1–O21	92.7(1)	91.4(4)	93.8(3)	92.2(4)
N11–Ni1–O11	93.3(1)	91.7(4)	91.5(4)	93.2(4)
N11–Ni1–O21	89.9(1)	88.5(5)	89.0(5)	90.5(5)
O11–Ni1–O21	176.3(1)	178.1(5)	178.3(4)	176.3(4)

N11) and uncoordinated oxygen atoms of the phosphonates (O23 and O12, respectively) with intermediate lengths ($d_{N\cdots O}$ ~ 2.9 Å). The complex molecules are connected in infinite chains *via* a very short intermolecular hydrogen bond between one –OH group of the double-protonated phosphonate (O12) and the non-protonated oxygen atom of the second pendant phosphonate of a neighbouring molecule (O23$^{\#}$) with O12···O23$^{\#}$ separation of 2.41 Å. Similar short hydrogen bonds connecting the neighbouring units were observed also in the structures of *trans-O,O*-[Co(Hte2p^{1,8})]·6H_2O (ref. 38) and *trans-O,O*-[Cu(H_2te2p^{1,8})]·2H_2O (ref. 23) complexes. A further hydrogen bond network is formed between protonated phosphonates, the water solvate and the chloride anions.

Crystal structure of {*trans-O,O*-[Ni(H_2L)]}$_3$·5H_2O. The independent unit is formed by three complex molecules and five solvate water molecules. Coordination spheres of all nickel(II) ions are very similar to each other and to the previous complex, with four macrocycle nitrogen atoms in equatorial positions (with configuration **III**) and two phosphonate oxygen atoms in axial positions (Fig. S1†). Coordination bond lengths have common values for these types, with slightly longer bonds (by ~0.06 Å) between the central ion and the tertiary amino groups compared to the secondary ones (Table 1). In summary, the low quality of the diffraction data led to high uncertainties of atom positions and high anisotropic displacement parameters. However, the complex stereochemistry was unambiguously determined.

Ligand protonation

The first two dissociation constants of azacycle amino groups having phosphonic acid pendant arms are usually very high (pK_a ~13),[39] *i.e.* the values lie out of the region of potentiometric titrations and, thus, they cannot be determined by this method. Therefore, ^{31}P NMR titration was employed to obtain the values of log β_{11} and log β_{21}. However, only the value of log β_{21} constant could be fitted. This fact can be attributed to lower precision of data due to non-constant ionic strength at –log[H$^+$] > 12.5 and/or a relatively low number of data points (comparing to potentiometry). In addition, such behaviour can point to a "reverse order" of protonation (dissociation) constants due to the presence of an intramolecular hydrogen bond (for more discussion see Fig. S2†).[22] The presence of strong hydrogen bonds in the system is evidenced from the appearance of ^{1}H NMR spectra at different solution pH. The signals are broad in pH region 3–13 (where the macrocyclic amine groups bind just two protons, Fig. S3†). The corresponding overall protonation constant log β_{21} is higher than that observed for cyclam[40] and tetraacetate derivative H_4teta,[41] as it is typical for aminomethylphosphonic acids.[39,42,43] The third and fourth protons are attached to oxygen atoms of the phosphonate moieties, with the values of the dissociation constants (pK_a 6.56 and 5.19) typical for such dissociations.[39,42,43]

The final values of protonation (dissociation) constants were obtained from simultaneous fitting of both ^{31}P NMR and potentiometry data and are compiled in Table 2. The corresponding distribution diagram is given in Fig. S4.†

Stability of complex species

In all systems studied, the ligand forms 1 : 1 complex species which can be additionally mono- and double-protonated. In the systems with Pb(II), a triple-protonated species was also detected in a low abundance at –log[H$^+$] 4–7. The results are compiled in Table 3.

Table 2 Protonation[a] constants of H_4te2p^{1,4} and dissociation[b] constants of H_4te2p^{1,4} and related ligands (Chart 1; 25.0 °C, I(KNO_3) = 0.1 M)

	log β_h[a]		pK_a[b]						
h	H_4te2p^{1,4}	Equilibrium[c]	H_4te2p^{1,4}	Cyclam [40]	H_2te1p [21]	H_1te1a [14a]	H_4te2p^{1,8} [22]	H_8tetp [44]	H_4teta [41]
1	—	HL ⇌ H$^+$ + L	—	11.29	12.49	12.18	—	—	10.58
2	*25.72(3)*[d]	H_2L ⇌ H$^+$ + HL	*25.72*[d]	10.19	11.76	10.87	*26.41*[d]	*25.28*[d]	10.17
3	32.28(1)	H_3L ⇌ H$^+$ + H_2L	6.56	1.61	6.05	3.01	6.78	8.85	4.09
4	37.47(1)	H_4L ⇌ H$^+$ + H_3L	5.19	1.91	2.42	< 2	5.36	7.68	3.35
5	39.77(1)	H_5L ⇌ H$^+$ + H_4L	2.30	—	—	< 2	1.15	6.23	—
6	—	H_6L ⇌ H$^+$ + H_5L	—	—	*2.16*[d]	—	—	5.33	—

[a] β_h = [H_hL]/([H]^h × [L]). [b] K_a = ([H] × [H_{n-1}L])/[H_nL]. [c] Charges of species are omitted for clarity. [d] Values *in italics* correspond to protonation/dissociation over two steps.

Table 3 Stability constants of complexes of H_4te2p[1,4] with selected metal ions (25.0 °C, $I(KNO_3) = 0.1$ M)

Cation	h	l	m	$\log \beta_{hlm}$ [a]	pK_a [b]
Ni(II)	0	1	1	21.92(6)	—
	1	1	1	28.06(6)	6.14
	2	1	1	33.18(3)	5.12
Cu(II)	0	1	1	27.21(3)	—
	1	1	1	34.02(3)	6.81
	2	1	1	38.93(2)	4.91
Zn(II)	0	1	1	20.16(3)	—
	1	1	1	27.04(1)	6.88
	2	1	1	31.47(4)	4.43
Cd(II)	0	1	1	17.03(2)	—
	1	1	1	24.83(1)	7.80
	2	1	1	29.75(3)	4.92
Pb(II)	0	1	1	12.85(4)	—
	1	1	1	23.05(3)	10.20
	2	1	1	29.44(3)	6.39
	3	1	1	34.73(9)	5.29
Ca(II)	0	1	1	3.42(5)	—
	1	1	1	15.34(9)	11.92
	2	1	1	27.46(5)	12.12

[a] $\beta_{hlm} = [H_hL_lM_m]/([H]^h \times [L]^l \times [M]^m)$. [b] $K_a = ([H] \times [M(H_{h-1}L)])/[M(H_hL)]$.

In the case of calcium(II), the value of $\log \beta_{011}$ is low, pointing to a very low abundance of the complex species in solution (Fig. S5†). The initially formed diprotonated complex $[Ca(H_2L)]$ probably loses protons from nitrogen atoms of the macrocycle, as can be seen from the pK_as of 12.12 and 11.92. Thus, the metal ion is probably coordinated only by the oxygen atoms of the phosphonate pendant moiety in double-protonated complex, and the macrocyclic part starts to coordinate only after the full deprotonation in very alkaline solutions. Similar coordination behaviour was found also for H_4te2p[1,8] (ref. 45) and H_2te1p (ref. 21).

In the case of lead(II) complexation, the value of $\log \beta_{011}$ is much higher than that found for Ca(II), and the abundances of complex species are also higher. Free lead(II) ion is not present in the equimolar mixture above pH ~8 (Fig. S6†). The complexation starts with formation of triprotonated complex $[Pb(H_3L)]^+$, which has maximal abundance at pH ~5. According to its pK_a (5.29), it loses a proton probably from the phosphonate pendant arm. The second pK_a (6.39) is also comparable to the values corresponding to phosphonate moieties, but the last one is much higher (10.20). It suggests two possible explanations. In the first case, two oxygen atoms (one of each pendant arm) are protonated as well as one nitrogen atom of the macrocycle in the triple-protonated complexes. In such case, at least other nitrogen atoms of the macrocycle should be involved in metal coordination in the $[Pb(H_2L)]$ species; but such a species exists even at pH below 5, and such coordination of the macrocycle nitrogen atom would be very unusual. The second possibility suggests that the metal ion is bound only by pendant arms in the triple-protonated complex and two macrocycle nitrogen atoms are still protonated. In this case, due to a higher affinity of Pb(II) for nitrogen coordination (compared to the Ca(II) complex), the pK_a values corresponding to the $[Pb(H_2L)]$ species would be lowered by several orders of magnitude compared to those of the free ligand. Furthermore, as the X-ray diffraction study of the solid phase formed from Pb(II)–H_4te2p[1,8] system at pH ~6 revealed Pb(II) coordination by only phosphonate groups and protonation of two nitrogen atoms.[45] So, the second alternative of proton locations seems to be correct.

In the systems with transition metal ions (Ni(II), Cu(II), Zn(II) and Cd(II)), the high values of the $\log \beta_{011}$ and the values of the pK_a clearly show that all four nitrogen atoms are coordinated in all complex species, and the coordination sphere is probably closed by at least one phosphonate pendant arm. The protonation of these complexes takes place on the non-coordinated phosphonate oxygen atoms (see also above for the crystal structure of Ni(II) complex). The first protonation occurs with pK_as slightly higher (6.81 for Cu(II), 6.88 for Zn(II) and 7.80 for Cd(II)) or slightly lower (6.14 for Ni(II)) than the corresponding pK_a value of the free ligand (6.56). The second protonations occur with lower pK_as compared to that of the free ligand. The similar drop of pK_a values of the phosphonate pendant arm upon coordination was observed also for analogous complexes of H_4te2p[1,8] (ref. 23, 45) and H_2te1p (ref. 21).

The highest stability was found for the Cu(II) complex, as it commonly reflects the Irving–Williams trend and, in addition, the optimal size of the cyclam ring for this cation. The complexation of Cu(II) starts in the acidic region and the metal ion is fully encapsulated in the complex below pH ~3 (Fig. 4). The selectivity of the ligand for Cu(II) is very high as the difference between corresponding stability constants is about seven and five orders of magnitude for the Cu/Zn and Cu/Ni pairs, respectively. These facts are very promising for a potential analytical or radiopharmaceutical use. The calculated distribution diagrams of other studied systems are given in the ESI (Fig. S7–S9).†

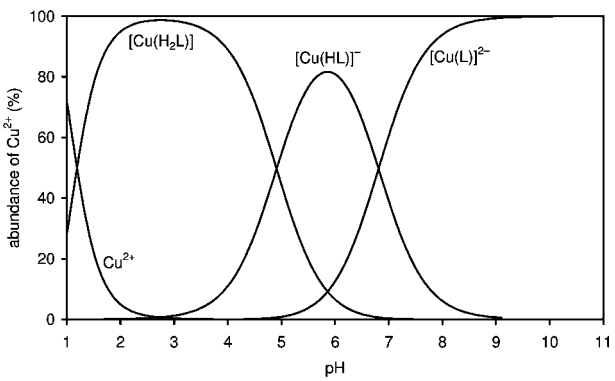

Fig. 4 Distribution diagram for the Cu(II)–H_4te2p[1,4] system ($c_L = c_{Cu} = 0.004$ M).

From the comparison of stability constants determined for the title ligand and the related compounds (Table 4), it is clear that the cyclam skeleton substituted by several pendant arms based on phosphorus acid is a good choice as the ligands have a high selectivity of copper(II) complexation. In the H_4te2p[1,4] case, the complexation of copper(II) is more selective compared to its 1,8-isomer[23] and the monophosphonate derivative H_2te1p.[21] Furthermore, the complexation reaction is relatively fast (similarly to the 1,8-isomer) in slightly acid solutions, and much faster than that of the monophosphonate ligand, H_2te1p,[21] and cyclam[46] itself; it points to an acceleration effect of more phosphonate pendant arms. The concentration of free Cu(II) ion in the Cu(II)–H_4te2p[1,4] system (expressed as $pCu = -\log [Cu(II)]$ calculated for an equimolar ligand–Cu(II) mixture) is about one order of magnitude lower than that for the 1,8-isomer and closer to values for other ligands with coordinating pendant arms (Table 4). However,

Table 4 Comparison of stability constants ($\log \beta_{011}$) of the [M(L)] complex species formed with $H_4te2p^{1,4}$ and related ligands

Ligand	Ni(II)	Zn(II)	Cd(II)	Pb(II)	Ca(II)	Cu(II)	pCu[a]
$H_4te2p^{1,4}$	21.92	20.16	17.03	12.85	3.45	27.21	9.4
$H_4te2p^{1,8}$ [b]	21.99	20.35	17.89	14.96	5.26	25.40	8.1
H_2te1p[c]	—	21.03	15.91	—	3.07	27.34	10.1
Cyclam[d]	22.2	15.2	11.3	10.9	—	28.1	11.9
H_4teta[d]	19.91	16.62	18.25	14.3	8.42	21.74	9.1
H_8tetp	—	17.6[e]	16.7[e]	15.5[e]	—	25.99[f]	8.4

[a] Calculated for $-\log[H^+] = 7.4$ and $c_L = c_M = 0.004$ M. [b] Ref. 23, 45. [c] Ref. 21. [d] Ref. 47. [e] Ref. 48. [f] Ref. 41*b*.

the lower pCu values for the phosphorus-containing ligands are mainly given by the very high overall basicity of the ligands. The kinetics of complex formation and dissociation is under study.

Conclusions

The title ligand, $H_4te2p^{1,4}$, forms extremely stable complex with Cu(II) ($\log \beta(CuL) = 27.21$) with a high selectivity of complexation over other metal ions (*e.g.* $\log \beta(ZnL) = 20.16$, $\log \beta(NiL) = 21.92$). The complexation of copper(II) proceeds relatively fast as it could be determined by conventional potentiometric titration. It makes the ligand promising as the parent chelator for possible radiopharmaceutical applications. The ligand forms octahedral *trans-O,O* Ni(II) complexes with the most stable macrocycle configuration **III**. From acid solution, the crystals of *trans-O,O*-$[Ni(H_3L)]Cl{\cdot}H_2O$ were isolated with mono- and double-protonated phosphonate pendant arms. The isolation of such species points to a high inertness of the complex. From neutral solutions, the crystals of $\{$*trans-O,O*-$[Ni(H_2L)]\}_3{\cdot}5H_2O$ were isolated where each pendant arm is monoprotonated.

Acknowledgements

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Notes and references

1 (*a*) A. E. Martell and R. D. Hancock, *Metal Complexes in Aqueous Solutions*, Plenum Press, New York, 1996; (*b*) K. P. Wainwright, *Coord. Chem. Rev.*, 1997, **166**, 35–90; (*c*) S. F. Lincoln, *Coord. Chem. Rev.*, 1997, **166**, 255–289; (*d*) L. F. Lindoy, *Adv. Inorg. Chem.*, 1998, **45**, 75–125; (*e*) R. Delgado, V. Félix, L. M. P. Lima and D. W. Price, *Dalton Trans.*, 2007, 2734–2745.

2 M. Meyer, V. Dahaoui-Ginderey, C. Lecomte and R. Guilard, *Coord. Chem. Rev.*, 1998, **178–180**, 1313–1405.

3 X. Liang and P. J. Sadler, *Chem. Soc. Rev.*, 2004, **33**, 246–266.

4 E. De Clercq, *Nat. Rev. Drug Discovery*, 2003, **2**, 581–587.

5 (*a*) G. J. Bridger and R. T. Skerlj, *Adv. Antiviral Drug Des.*, 1999, **3**, 161–229; (*b*) X. Liang, J. A. Parkinson, M. Weishaupl, R. O. Gould, S. J. Paisey, H. S. Park, T. M. Hunter, C. A. Blindauer, S. Parsons and P. J. Sadler, *J. Am. Chem. Soc.*, 2002, **124**, 9105–9112; (*c*) L. O. Gerlach, J. S. Jakobsen, K. P. Jensen, M. R. Rosenkilde, R. T. Skerlj, U. Ryde, G. J. Bridger and T. W. Schwartz, *Biochemistry*, 2003, **42**, 710–717.

6 (*a*) S. Liu and D. S. Edwards, *Bioconjugate Chem.*, 2001, **12**, 7–34; (*b*) S. Liu and D. S. Edwards, *Top. Curr. Chem.*, 2002, **222**, 259–278; (*c*) *Handbook of Radiopharmaceuticals. Radiochemistry and Applications*, ed. M. J. Welch and C. S. Redvanly, Wiley, Chichester, UK, 2003; (*d*) S. Liu, *Chem. Soc. Rev.*, 2004, **33**, 445–461; (*e*) W. P. Li, L. A. Meyer and C. J. Anderson, *Top. Curr. Chem.*, 2005, **252**, 179–192.

7 (*a*) R. B. Wilder, G. L. DeNardo and S. J. DeNardo, *J. Clin. Oncol.*, 1996, **14**, 1383–1400; (*b*) I. Novak-Hofer and P. A. Schubiger, *Eur. J. Nucl. Med. Mol. Imaging*, 2002, **29**, 821–830; (*c*) S. V. Smith, *J. Inorg. Biochem.*, 2004, **98**, 1874–1901; (*d*) X. Sun and C. J. Anderson, *Methods Enzymol.*, 2004, **386**, 237–261; (*e*) T. J. Wadas, E. H. Wong, G. R. Weisman and C. J. Anderson, *Curr. Pharm. Des.*, 2007, **13**, 3–16.

8 M. K. Moi, C. F. Meares, M. J. McCall, W. C. Cole and S. J. DeNardo, *Anal. Biochem.*, 1985, **148**, 249–253.

9 (*a*) A. Riesen, M. Zehnder and T. A. Kaden, *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.*, 1988, **44**, 1740–1742; (*b*) E. Espinosa, M. Meyer, D. Berard and R. Guilard, *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.*, 2002, **58**, m119-m121; (*c*) J. D. Silversides, C. C. Allan and S. J. Archibald, *Dalton Trans.*, 2007, 971–978.

10 L. A. Bass, M. Wang, M. J. Welch and C. J. Anderson, *Bioconjugate Chem.*, 2000, **11**, 527–532.

11 (*a*) F. I. Belskii, Y. M. Polikarpov and M. I. Kabachnik, *Usp. Khim.*, 1992, **61**, 415–455; (*b*) C. F. G. C. Geraldes, M. P. M. Marques, B. de Castro and E. Pereira, *Eur. J. Inorg. Chem.*, 2000, 559–565; (*c*) X. Sun, M. Wuest, Z. Kovacs, A. D. Sherry, R. Motekaitis, Z. Wang, A. E. Martell, M. J. Welch and C. J. Anderson, *JBIC, J. Biol. Inorg. Chem.*, 2003, **8**, 217–225.

12 (*a*) I. Lázár, A. D. Sherry, R. Ramasamy, E. Brücher and R. Kiraly, *Inorg. Chem.*, 1991, **30**, 5016–5019; (*b*) K. Bazakas and I. Lukeš, *J. Chem. Soc., Dalton Trans.*, 1995, 1133–1137; (*c*) J. Rohovec, M. Kývala, P. Vojtíšek, P. Hermann and I. Lukeš, *Eur. J. Inorg. Chem.*, 2000, 195–203; (*d*) P. Lubal, M. Kývala, P. Hermann, J. Holubová, J. Rohovec, J. Havel and I. Lukeš, *Polyhedron*, 2001, **20**, 47–55.

13 (*a*) E. K. Barefield and F. Wagner, *Inorg. Chem.*, 1973, **12**, 2435–2439; (*b*) L. Hertli and T. A. Kaden, *Helv. Chim. Acta*, 1974, **57**, 1328–1333.

14 (*a*) M. Studer and T. A. Kaden, *Helv. Chim. Acta*, 1986, **69**, 2081–2086; (*b*) D. Tschudin, A. Basal and T. A. Kaden, *Helv. Chim. Acta*, 1988, **71**, 100–106.

15 D. Tschudin, A. Riesen and T. A. Kaden, *Helv. Chim. Acta*, 1989, **72**, 313–319.

16 (*a*) I. M. Helps, D. Parker, J. Chapman and G. Ferguson, *J. Chem. Soc., Chem. Commun.*, 1988, 1094–1095; (*b*) J. Chapman, G. Ferguson, J. F. Gallagher, M. C. Jennings and D. Parker, *J. Chem. Soc., Dalton Trans.*, 1992, 345–353.

17 (*a*) A. Comparone and T. A. Kaden, *Helv. Chim. Acta*, 1998, **81**, 1765–1772; (*b*) L. Siegfried, A. Comparone, M. Neuburger and T. A. Kaden, *Dalton Trans.*, 2005, 30–36.

18 C. Bucher, E. Duval, J.-M. Barbe, J.-N. Verpeaux, C. Amatore and R. Guilard, *C. R. Acad. Sci., Ser. IIc: Chim.*, 2000, **3**, 211–222.

19 (*a*) A. E. Goeta, J. A. K. Howard, D. Maffeo, H. Puschmann, J. A. G. Williams and D. S. Yufit, *J. Chem. Soc., Dalton Trans.*, 2000, 1873–1880; (*b*) A. S. Batsanov, A. E. Goeta, J. A. K. Howard, D. Maffeo, H. Puschmann and J. A. G. Williams, *Polyhedron*, 2001, **20**, 981–986.

20 H. Kurosaki, C. Bucher, E. Espinosa, J.-M. Barbe and R. Guilard, *Inorg. Chim. Acta*, 2001, **322**, 145–149.

21 S. Füzerová, J. Kotek, I. Císařová, P. Hermann, K. Binnemans and I. Lukeš, *Dalton Trans.*, 2005, 2908–2915.

22 J. Kotek, P. Vojtíšek, I. Císařová, P. Hermann, P. Jurečka, J. Rohovec and I. Lukeš, *Collect. Czech. Chem. Commun.*, 2000, **65**, 1289–1316.

23 J. Kotek, P. Lubal, P. Hermann, I. Císařová, I. Lukeš, T. Godula, I. Svobodová, P. Táborský and J. Havel, *Chem.–Eur. J.*, 2003, **9**, 233–248.

24 (*a*) I. Svobodová, P. Lubal, P. Hermann, J. Kotek and J. Havel, *Microchim. Acta*, 2004, **148**, 21–26; (*b*) I. Svobodová, P. Lubal, P. Hermann, J. Kotek and J. Havel, *J. Inclusion Phenom. Macrocyclic Chem.*, 2004, **49**, 11–15.

25 T. Vitha, J. Kotek, J. Rudovský, V. Kubíček, I. Císařová, P. Hermann and I. Lukeš, *Collect. Czech. Chem. Commun.*, 2006, **71**, 337–367.

26 F. Bellouard, F. Chuburu, N. Kervarec, L. Toupet, S. Triki, Y. LeMest and H. Handel, *J. Chem. Soc., Perkin Trans. 1*, 1999, 3499–3505.

27 (*a*) E. K. Barefield, *Inorg. Chem.*, 1972, **11**, 2273–2274; (*b*) I. Meunier, A. K. Mishra, B. Hanquet, P. Cocolios and R. Guilard, *Can. J. Chem.*, 1995, **73**, 685–695.

28 (*a*) Z. Otwinovski and W. Minor, *HKL Denzo and Scalepack Program Package*, Nonius BV, Delft, 1997; (*b*) Z. Otwinovski and W. Minor, *Methods Enzymol.*, 1997, **276**, 307–326.

29 A. Altomare, M. C. Burla, M. Camalli, G. Cascarano, C. Giacovazzo, A. Guagliardi and G. Polidori, *J. Appl. Crystallogr.*, 1994, **27**, 435.

30 G. M. Sheldrick, *SHELXL-97, Program for refinement of crystal structures*, University of Göttingen, Germany, 1997.

31 P. Coppens, in *Crystallographic Computing*, ed. F. R. Ahmed, S. R. Hall and C. P. Huber, Munksgaard, Copenhagen, 1970, pp. 255–270.

32 C. F. Baes, Jr. and R. E. Mesmer, *The Hydrolysis of Cations*, Wiley, New York, 1976.
33 M. Kývala and I. Lukeš, *Chemometrics '95*, Pardubice, Czech Republic, 1995, p. 63, full version of *OPIUM program package* is available (free of charge) at http://www.natur.cuni.cz/~kyvala/opium.html.
34 K. Popov, H. Ronkkomaki and L. H. J. Lajunen, *Pure Appl. Chem.*, 2006, **78**, 663–675.
35 B. Bosnich, C. K. Poon and M. L. Tobe, *Inorg. Chem.*, 1965, **4**, 1102–1108.
36 (*a*) M. A. Donnelly and M. Zimmer, *Inorg. Chem.*, 1999, **38**, 1650–1658; (*b*) T. M. Hunter, S. J. Paisey, H.-S. Park, L. Cleghorn, A. Parkin, S. Parsons and P. J. Sadler, *J. Inorg. Biochem.*, 2004, **98**, 713–719.
37 J. Kotek, P. Vojtíšek, I. Císařová, P. Hermann and I. Lukeš, *Collect. Czech. Chem. Commun.*, 2001, **66**, 363–381.
38 J. Kotek, P. Hermann, I. Císařová, J. Rohovec and I. Lukeš, *Inorg. Chim. Acta*, 2001, **317**, 324–330.
39 I. Lukeš, J. Kotek, P. Vojtíšek and P. Hermann, *Coord. Chem. Rev.*, 2001, **216–217**, 287–312.
40 R. D. Hancock, R. J. Motekaitis, J. Mashishi, I. Cukrowski, J. H. Reibenspies and A. E. Martell, *J. Chem. Soc., Perkin Trans. 2*, 1996, 1925–1929.
41 (*a*) S. Chaves, R. Delgado, J. J. R. Frausto and Da Silva, *Talanta*, 1992, **39**, 249–254; (*b*) R. Delgado, J. Costa, K. P. Guerra and L. M. P. Lima, *Pure Appl. Chem.*, 2005, **77**, 569–579.
42 T. Kiss and I. Lázár, in *Aminophosphonic and Aminophosphinic Acids. Chemistry and Biological Activity*, ed. V. P. Kukhar and H. R. Hudson, Wiley, Chichester, UK, 2000, ch. IX, pp. 285–326.
43 K. Popov, H. Rönkkömäki and L. H. J. Lajunen, *Pure Appl. Chem.*, 2001, **73**, 1641–1677.
44 (*a*) R. Delgado, L. C. Siegfried and T. A. Kaden, *Helv. Chim. Acta*, 1990, **73**, 140–148; (*b*) F. Marques, L. Gano, M. P. Campello, S. Lacerda, I. Santos, L. M. P. Lima, J. Costa, P. Antunes and R. Delgado, *J. Inorg. Biochem.*, 2006, **100**, 270–280.
45 I. Svobodová, P. Lubal, J. Plutnar, J. Havlíčková, J. Kotek, P. Hermann and I. Lukeš, *Dalton Trans.*, 2006, 5184–5197.
46 R. J. Motekaitis, B. E. Rogers, D. E. Reichert, A. E. Martell and M. J. Welch, *Inorg. Chem.*, 1996, **35**, 3821–3827.
47 (*a*) A. E. Martell and R. M. Smith, *Critical Stability Constants*, Plenum Press, New York, 1974–1989, vol. 1–6; (*b*) *NIST Standard Reference Database 46 (Critically Selected Stability Constants of Metal Complexes)*, Version 7.0, 2003, distributed by NIST standard Reference Data, Gaithersburg, MD 20899, USA.
48 S. A. Pisareva, F. I. Belskii, T. Ya. Medved and M. I. Kabachnik, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1987, 413–417.

