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Formation and decomplexation kinetics of copper(II) complexes with cyclen derivatives having mixed carboxylate and phosphonate pendant arms†

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The kinetic properties of Cu(II) complexes of H_4dota and its analogues with one (H_5do3ap), two in the 1,7-position (*trans*-H_6do2a2p), three (H_7doa3p) and four (H_8dotp) phosphonic acid pendant arms were investigated. The formation of a Cu(II) complex with H_4dota, *trans*-H_6do2a2p and H_8dotp at a slightly acidic pH is faster for the phosphonic acid derivatives than for H_4dota, but with no simple dependence on the number of $-CH_2PO_3H_2$ substituents (*trans*-H_6do2a2p > H_8dotp > H_4dota; pH 4–6). Relative differences in the reactivity among the differently protonated species (H_nL^{x-}) of the same ligand are successively decreased with the more phosphonic acid groups in the ligand. The faster complexation is probably caused by the higher ability of phosphonates to bind the metal ion and/or to assist in the transfer of protons from the ring amine groups to the bulk water. The acid-assisted decomplexation kinetics of the complexes was followed in highly acidic solutions ($[H^+]$ = 0.01–5 M) and at different temperatures (15–70 °C) to determine the activation parameters of the reaction. The kinetic inertness of the Cu(II) complexes follows the order: H_4dota > H_5do3ap > *trans*-H_6do2a2p > H_7doa3p > H_8dotp. To obtain information on the influence of additional pendant arms, analogous data were obtained for *trans*-H_2do2a. The ligand is less reactive than H_4dota, but the kinetic inertness of its Cu(II) complex is similar to that of the H_4dota complex. As it was considered that the published thermodynamics data on the Cu(II)–H_8dotp system are probably incorrect, the system was re-investigated. It showed a very high stability for the $[Cu(dotp)]^{6-}$ species and the easy formation of several Cu_2L species in the presence of an excess of the metal ion. Also, the structure of the $(H_6doa3p)^-$ anion in the solid state was determined. These experimental data demonstrate that the substitution of acetic acid pendant arms by methylphosphonic acid ones in H_4dota-like ligands increases the rate of complexation but significantly decreases the kinetic inertness of the Cu(II) complexes.

Introduction

Tetraazamacrocyclic ligands with coordinating pendant arms have been studied intensively for a number of years due to their utilization in medicine and molecular biology, *e.g.* as contrast agents in magnetic resonance imaging (MRI)[1] or as metal radioisotope carriers for diagnostic and/or therapeutic purposes.[2–4] Generally, metal complexes intended for applications in medicine should exhibit some specific properties, namely high thermodynamic stability and kinetic inertness, desired hydrophilicity/hydrophobicity, *etc*. To achieve the desired biodistribution/pharmacokinetics of the metal complexes, conjugation to a targeting molecule, such as (oligo)peptides, monoclonal antibodies or their fragments, sugars, *etc*., is necessary, with ligands bearing a reactive group (bifunctional chelators, BFC)[5] usually employed for such conjugations. For metal radioisotopes, the rate of complexation in

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†Electronic supplementary information (ESI) available: Distribution diagrams and spectral profiles of species present in the Cu(II)–H_4dota, Cu(II)–*trans*-H_6do2a2p, Cu(II)–H_8dotp systems; primary titration data for the Cu(II)–H_8dotp system; additional formation kinetics data and selected figures; additional acid-assisted decomplexation kinetics data and selected figures; experimental and additional data for the solid-state structure of $K(H_6doa3p)\cdot)$, $3H_2O$ and a figure of its crystal packing. CCDC 1469446. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c6dt01127f

very dilute solutions is also an important parameter.[6] In general, metal complexes of macrocyclic ligands are much more kinetically inert than the complexes of open-chain ligands but their formation is rather slow. The properties of complexes have to be carefully balanced through ligand design and these investigations comprise a vital multidisciplinary field where coordination chemistry plays an important role.

In nuclear medicine, a number of metal radioisotopes have been suggested: $^{44/47}$Sc, $^{61/64/67}$Cu, $^{68/67}$Ga, ^{90}Y, ^{99m}Tc, ^{111}In, ^{153}Sm, ^{166}Ho, ^{177}Lu, $^{186/188}$Re, ^{213}Bi, *etc*. Among these, copper radioisotopes bring a number of advantages as they are useful both for diagnosis ($^{61/64}$Cu, β^+ emitters for PET imaging) and therapy (^{67}Cu, β^- emitter) and their half-life match the pharmacokinetics of different targeting molecules, *e.g.* oligopeptides or antibody fragments. Research on ligands for copper radioisotopes has continued over the last two decades[2,3,7] but an optimal ligand family has not been found, until now. However, due to their commercial availability, bifunctional H_4dota-like ligands are the most common chelators used in ^{64}Cu radiopharmaceuticals research, despite them not having optimal complexation properties towards divalent copper.

The properties of macrocyclic ligands can be tuned through the size of the macrocyclic ring, the ring donor atoms and/or the nature of the coordinating pendant arms. The most commonly studied ligands are those based on polyazamacrocycles, namely 1,4,7-triazacyclononane (tacn), 1,4,7,10-tetraazacyclododecane (cyclen) and 1,4,8,11-tetrazacyclotetradecane (cyclam). The most common pendant arm in these ligands is the acetic acid moiety (where the prototype ligand is *e.g.* H_4dota, Fig. 1), though acetamide, propionic acid, alcoholic, methylphosphonic/phosphinic acid and various heterocycle pendant arms have also been suggested. Among these, the acid–base and coordination properties of phosphorus acid groups are the most similar to the properties of the carboxylic group and, therefore, methylphosphonic or methylphosphinic acid pendants are a common choice.[8,9] The groups are tetrahedral and more acidic, harder and larger, as well as more hydrophilic than the carboxylic group. For phosphinic acid derivatives, their properties could be further tuned through changing the phosphorus atom substituents and, in addition, by preparing bifunctional chelators with a reactive side group on the phosphorus atom.[10–13] With regard the other pendant arms, the heterocycle-containing ones (*e.g.* methylpicolinate) highly improve Cu(II) binding into the macrocyclic cavity and the complex properties.[14,15] Some kinetic data on Cu(II) complexes of the cyclen/cyclam derivatives with pendant arms have been published and the investigations on the complexes of ligands with acetic acid,[16–18] methylphosphonic/phosphinic acid[13,18–23] or heterocyclic pendant arms[14,15] have shown that the kinetic properties are highly influenced by the nature of the pendant arms. Generally, Cu(II) complex formation is accelerated when phosphorus acid or picolinate pendant arms are present, and this is also true with non-carrier-added ^{64}Cu for cyclam[13,23,24] as well as for cross-bridged cyclam derivatives;[25] in addition, these groups are considered the best pendant arms for radiolabelling.

With the lanthanide(III) complexes of H_4dota-like ligands, it has been shown that the presence of one phosphorus acid pendant accelerates complexation, but it is decelerated with more phosphonic acid pendants.[11,26,27b] In these complexes, the influence of the number of phosphorus acid pendants on decomplexation is not clear.[27–31] To obtain analogous data for Cu(II) complexes of cyclen-based ligands, we decided to compare the kinetic behaviour of the complexes with H_4dota and its analogues with an increasing number of methylphosphonic acid pendant arms: one (H_5do3ap), two in the 1,7-position (*trans*-H_6do2a2p), three (H_7do1a3p) and four (H_8dotp); see Fig. 1. The coordination chemistry of the symmetric ligands, H_4dota and H_8dotp, has previously been investigated in a number of studies over a long time; however, for Cu(II), only the dissociation kinetics of its H_4dota and H_5do3ap complexes[18] and partially also the formation kinetics with H_4dota[16] have been studied. The copper(II) complexes of some methylphosphonic acid cyclen derivatives have also been studied but no kinetic data has been published[32,33] except for our recent work on tris(methylphosphonic acid) cyclen derivatives.[19]

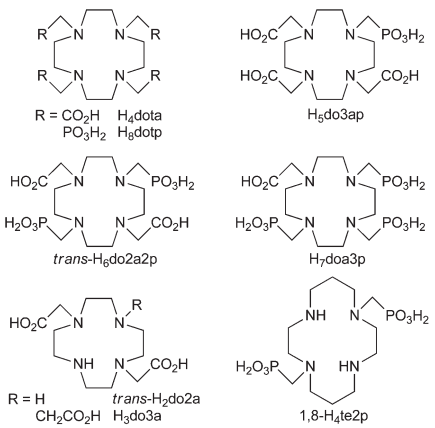


Fig. 1 Structures of the discussed ligands.

Experimental

The ligands were synthesized according to the published procedures for H_4dota,[34] H_5do3ap,[35] *trans*-H_6do2a2p,[28] H_7doa3p,[31] H_8dotp[33] and *trans*-H_2do2a.[36] The ligands were used in a zwitterionic form, and any inorganic acids (originating from the ligand syntheses), if present, were removed by ion exchangers. $CuCl_2{\cdot}2H_2O$ was purchased from Fluka and its stock solution was standardized chelatometrically.[37] The other analytical grade chemicals were purchased from Lachema, Fluka or Merck, and were used as received. The Cu(II) complexes for the spectroscopic and dissociation kinetic studies were prepared by mixing $CuCl_2$ and the appropriate ligand stock solutions in a 1 : 1.2 molar ratio followed by stepwise neutralisation of the solutions to neutral pH.

All the kinetic experiments were done at 25.0 ± 0.1 °C or at the other temperatures mentioned in the text. The ionic strength of I = 0.1 M KCl or 5.0 M (Na,H)ClO_4 was used in the formation or dissociation kinetic studies, respectively. The kinetics was followed on a diode-array spectrophotometer HP 8453A (Hewlett Packard) and only the fast formation kinetics was measured by a stopped-flow technique (Applied Photophysics Instruments mixer joined with a diode-array detector, J&M and Applied Photophysics SX20). The formation kinetics of the Cu(II) complexes of *trans*-H_6do2a2p, H_7doa3p and H_8dotp were followed under pseudo-first-order conditions (c_{Cu} = (1–3.5) × 10^{-3} M, c_L = 1 × 10^{-4}–5 × 10^{-5} M) in the pH range 1–1.9. The initial-rate method was applied in the pH range 2.5–6.0 (c_{Cu} = 2.5 × 10^{-4} M, c_L = 5 × 10^{-4} M) in order to eliminate the possible formation of binuclear species in the course of the formation reaction. The dissociation kinetics of all the Cu(II) complexes was studied in 0.1–5 M $HClO_4$ with c_{CuL} = 1 × 10^{-4} M and in the temperature range 25–65 °C. The wavelength of the maximum of the absorption band in the range 315–325 nm was chosen to monitor the course of the formation/dissociation reactions. The experimental data were processed by PRO-K II (Applied Photophysics) and HP/Excel[38] software, giving identical results. The measured values of absorbance were corrected for the background analytical signal. Throughout the text, pH means $-\log[H^+]$.

UV-Vis spectra were measured (200–1100 nm, 25 °C) in aqueous solution at different pH. Electronic spectra of the individual species in the Cu–L systems were calculated from these data by OPIUM (a software package for the determination of stability constants, involving a module for spectrophotometric data treatment)[39] with knowledge of all the complex stability constants, *i.e.* knowing the abundance of individual species at each pH.

The ligand H_7doa3p was prepared as a hydrochloride.[31] For the kinetic experiments, HCl was removed by a strong cation exchanger (water elution), where the ligand is retarded more than HCl. Slow evaporation (*i.e.* weeks) of the first chromatographic fraction containing the ligand gave single crystals suitable for X-ray diffraction analysis.

Results and discussion

In-cage Cu(II) complexes in solution

To compare and interpret the kinetic data, the solution structures and thermodynamic stabilities of the complexes should be known. Speciation diagrams for the systems were calculated from literature data (Fig. S1, S3 and S5†). To obtain directly comparable data through the complex series, solution UV-Vis spectra of mononuclear Cu(II) complexes of H_4dota, *trans*-H_6do2a2p and H_8dotp were measured at various pH and the electronic spectra of the differently protonated species were calculated from the data (Fig. S2, S4 and S6; Table S1†).

The d–d spectra of Cu(II)–H_4dota/H_8dotp systems agree with the published data.[16,32,40] The absorption band maximum of $[Cu(dota)]^{2-}$ (λ_{max} ≈ 740 nm) was not changed with the addition of 1–2 protons to the $[Cu(H_ndota)]^{(2-n)}$ species. Their structures should be the same as that of $[Cu(H_2dota)]$ found in the solid state. There, the Cu(II) ion is bound in the ligand cavity (*in-cage* complex) with an N_2O_2 equatorial arrangement and with the two other amines in axial positions; in addition, the two acetate pendants are protonated and non-coordinated.[41] With an excess of Cu(II), a polymeric material with Cu_2L stoichiometry is formed in the solid state.[42] The d–d spectrum of $[Cu(dotp)]^{6-}$ (λ_{max} ≈ 650 nm) is not changed upon the addition of 1–3 protons. The spectrum of the $[Cu(H_4dotp)]^{2-}$ species is blue shifted (λ_{max} 628 nm). This could be caused by de-coordination of the axially bound phosphonate group upon the fourth protonation. In the solid state, the $[Cu(H_2O)(H_4dotp)]^{2-}$ species has a Cu(II) ion in the N_4-equatorial plane of a square-pyramid with a water molecule in the axial position and all phosphonate groups are monoprotonated and non-coordinated.[19] This structure was expected in acidic solutions from the UV-Vis (above) and EPR data[32] of the Cu(II)–H_8dotp system as they are similar to those of the $[Cu(H_2O)(cyclen)]^{2+}$ complex.[43] The UV band is changed with the complex protonation only in the Cu(II)–H_4dota system. Here, the N → Cu LMCT band (λ_{max} ≈ 350 nm) intensity is decreased and the O → Cu LMCT band (λ_{max} ≈ 290 nm) increased due to the lower electron density of the amine groups upon protonation of the non-coordinated carboxylates.

An intermediate behaviour was observed for the Cu(II)–*trans*-H_6do2a2p system (Fig. S2†), whereby the UV/Vis spectrum of the fully deprotonated $[Cu(do2a2p)]^{4-}$ anion resembles that of the $[Cu(dotp)]^{6-}$ species, suggesting the same solution structure. For the $[Cu(H_2do2a2p)]^{4-}$ anion (monoprotonated on each phosphonate group; see also below), the spectrum is very similar to that of the $[Cu(dota)]^{2-}$ where two acetates are bound to Cu(II). Therefore, the acetates should be coordinated to the Cu(II) ion and $-PO_3H^-$ groups, but having a rather low metal-ion binding affinity,[8] become free. The other protonated species may have different structures and/or they can be present in solution as a mixture of isomers.

Equilibrium studies of the Cu(II)–ligand systems have been published. The data seem to be reliable except those for the Cu(II)–H_8dotp system (see below). The ligand/complex protonation/stability constants for the Cu(II)–ligand systems are given in ESI (Tables S2 and S3†). Generally, complex stability is governed by ring amine basicity (defined as $\log\beta_2 = \log K_1 + \log K_2$), which is increased with a higher number of phosphonic acid pendant arms (Fig. S9†).[8] However, the Cu(II)–H_8dotp constants[33,44,45] do not fit well with this dependence and, therefore, they seem to be incorrect. The free ligand/complex phosphonate groups become monoprotonated in the pH range 5–8, while the carboxylates could be protonated in more acidic solutions. Copper(II) complexes of all the ligands are highly thermodynamically stable, as shown by $\log K_{CuL} > 20$. With Cu(II) excess, weak dinuclear complexes might be formed, whereby uncoordinated pendant arms probably bind the second metal ion (an *out-of-cage* complexation) and this ability is increasingly pronounced for the ligands with more phosphonate groups (Fig. S10†). The complex species protonated on

uncoordinated pendant arms are supposed to be fully thermodynamically stable in decomplexation kinetic experiments (see below) and the kinetically active species should only be those protonated on coordinated pendant arms. However in the Cu(II) complexes of such macrocyclic ligands, even diprotonated phosphonate or protonated carboxylate groups were found to be still coordinated to a central metal ion in the solid state.[20,46]

Cu(II)–H_8dotp system

The stability constants for the Cu(II)–H_8dotp system were redetermined as the values in the literature seem to be incorrect (see above). Experimental details on the determination and the found overall protonation/stability constants (Table S4†) as well as the complex species distribution diagrams for the L : Cu 1 : 1 and 1 : 2 molar ratios (Fig. S11) are given in ESI.†

First, the H_8dotp protonation constants were determined under our experimental conditions ($\log K_2$ – $\log K_7$: 12.75, 9.20, 8.01, 6.03, 5.18 and 1.74, respectively), and they were in excellent agreement with the published values.[47b] For treatment of the titration and kinetic data, the first protonation constant ($\log K_1$ 14.65) was taken from the literature[47b] as the protonation takes place outside of the potentiometric range. As complexes are almost quantitatively formed even in very acidic solutions (Fig. S11†), the titrations had to start at pH ≈ 1.4. The value of the stability constant obtained for the $[Cu(dotp)]^{6-}$ complex ($\log K_1$(CuL) 29.93) is several orders of magnitude higher than the published value[44] but fits well into the correlation mentioned above (Fig. S9†). The difference can be attributed to incorrect determination of the first ligand protonation constant in the original paper[44] as there are known problems with the determination of the H_8dotp protonation constants.[47] The first dissociation constant of the complex ($pK_{a4}(CuH_4L)$ 4.67; Table S5†) is much lower than that of the other ones ($pK_{a3-1}(CuH_{3-1}L)$ 6.43, 7.20 and 7.96, respectively) and its value is also lower than the corresponding constant in the free ligand. This indirectly points to metal-induced deprotonation of one $-PO_3H^-$ group in the free ligand connected with its coordination.

With an excess of metal ions, dinuclear complexes are very easily formed (Fig. S7 and S10†). In the pH region 3–5, their formation is slow (waiting time 2–2.5 min from each titration point) but still measurable by standard potentiometry. Protonated complexes as well as hydroxido-species with a Cu : L molar ratio of 2 : 1 had to be involved in the chemical model. The structure of the $[Cu_2(H_ndotp)]^{(8-n)-}$ (n = 0–2) species is probably not different from that of the $[Cu(H_ndotp)]^{6-n}$ (n = 0–4) complexes as the d–d transition λ_{max} of the dinuclear complexes are only slightly red shifted (to λ_{max} ≈ 660 nm). Their formation is accompanied by a solution colour change from deep blue to light blue due to the decrease in the absorption coefficients (Table S1†). The UV spectra of the dinuclear species (Fig. S8†) differ from those of the monomeric species. Bands observed at λ_{max} ≈ 225/≈330 nm for the dimeric species can be assigned to O → Cu/N → Cu LMCT bands analogously, as also suggested for the Cu(II) complexes of open-chain imino-bis(methylenephosphonic acid) ligands.[48] Similar observations have also been noticed for different Cu : L ratios in the Cu(II)–H_4dota system[41] (see above). Thus, the dinuclear complexes of H_8dotp should contain one *in-cage* bound Cu(II) ion, while the other one should interact only with oxygen atoms of non-coordinated phosphonate pendant arms. This is also supported by the values of the stepwise equilibrium constants for binding the second Cu(II) ion to the mononuclear complexes ($\log K_2(Cu_2L)$ 5.7–9.3, Table S5†). These values are similar to the values of the formation constants of monoprotonated Cu(II) complexes of simple imino-bis(methylenephosphonates), $\log K$ 6.7–10.7, where only oxygen atoms are coordinated.[49] The stability constants of all the Cu_2L complexes are reasonably correlated with the protonation constants of the uncoordinated pendant arms of the CuL complexes (Fig. S10†). This also indirectly supports the hypothesis that the second Cu(II) ion is bound by pendant arms only.

Formation kinetics

To follow the influence of the number and nature of pendant arms on the Cu(II) complex formation rate, a formation kinetics study was performed with *trans*-H_2do2a, H_4dota, *trans*-H_6do2a2p, H_7doa3p and H_8dotp, where the number of pendant arms was changed (*trans*-H_2do2a *vs.* H_4dota) and the number of phosphonic acids arms was increased (from H_4dota to H_8dotp). The formation of Cu(II) complexes takes place in a millisecond-to-hour timescale depending on the pH, leading the second-order formation constant to change by over five orders of magnitude (Fig. 2, S12 and S13†). Thus, both conventional (pH 1–3; Fig. S14–S19†) and stopped-flow (pH > 3; Fig. 2, S12 and S13†) techniques had to be employed.

Differently protonated ligand species, $(H_nL)^{n-m}$ (m = 4 and n = 2–5 for H_4dota, m = 6 and n = 2–6 for *trans*-H_6do2a2p, m = 7 and n = 4–5 for H_7doa3p, and m = 8 and n = 5–7 for H_8dotp) are present in the pH range 1–6 used for the investigations (Fig. S1, S3 and S5†) and they can participate in complexation reaction with Cu(II) ion to form the final *in-cage* complexes, $[Cu(H_nL)]^{x-}$ (Scheme 1). These, as well as less protonated

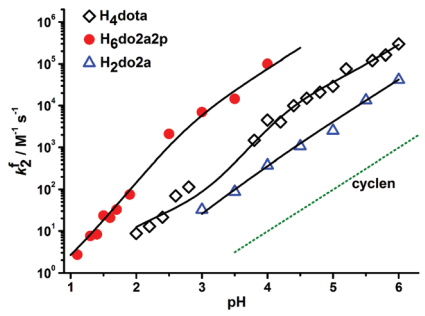
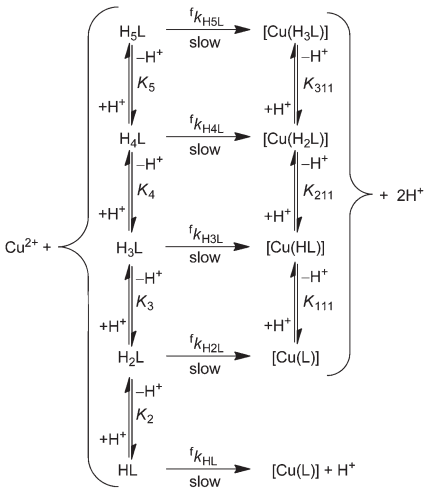


Fig. 2 Dependence of the second-order formation rate constants, $^fk_2$, for the Cu(II) complexes of H_4dota (◊), *trans*-H_6do2a2p (●) and *trans*-H_2do2a (Δ) on pH. The full-line curves were fitted through the experimental data according to eqn (1) and with the parameters given in Table 1. The curve for cyclen (dotted) was simulated on the basis of literature data.[50]



Scheme 1 Reaction scheme proposed for the formation of Cu(II) complexes with H_4dota, *trans*-H_6do2a2p, H_7doa3p or H_8dotp; the equilibrium constants *K* are stepwise ligand/complex protonation constants. Charges of the ligand/complex species are omitted for the sake of clarity.

ligand species, were involved in the fitting of the experimental data. No reverse reaction could be detected under the used experimental conditions (Fig. S15, S17 and S19†). The particular complex-forming reaction involving the protonated ligand species, $(H_nL)^{x-}$, was characterized by the partial second-order rate constant, ${}^{f}k_{H_nL}$, where these are related to the observed second-order rate constant ${}^{f}k_2$ through eqn (1):

$$k_2{}^{f} = \frac{\sum_{i=1}^{n} {}^{f}k_{H_iL}\beta_{p,i}[H^+]^i}{1 + \sum_{i=1}^{n} \beta_{p,i}[H^+]^i} \quad (1)$$

where $\beta_{p,i}$ are the overall protonation constants of the ligands (see Table S1†). The ${}^{f}k_2$ constant values measured at different pH were fitted as a function of solution acidity according to the general rate law, eqn (1). The protonated ligand species were considered in the fitting, and it was found that unique models with two to four reacting species according to the general Scheme 1 provided the best fit (Fig. 2, S12 and S13†); however, the less protonated species of the phosphonic acid-containing ligands cannot be involved. The corresponding ${}^{f}k_2$ constant values are given in Table 1. The ${}^{f}k_{H_nL}$ values obtained for H_4dota (n = 1–4: 1.4×10^9, 2.3×10^4, 764 and 37 M^{-1} s^{-1}, respectively) are comparable with those published by Wilkins *et al.*[16] (n = 1 and 2: 1.2×10^9 and 5×10^3 (estimated value) M^{-1} s^{-1}, respectively), which were measured in a very narrow pH range of 4.3–4.9.

As can be observed in Fig. 2 (cyclen *vs.* *trans*-H_2do2a/-H_4dota/*trans*-H_6do2a2p), the presence of pendant arms significantly accelerates formation of the Cu(II) complexes and this effect is observable throughout whole pH range. According to Fig. S12 and S13,† the most reactive ligands are *trans*-H_6do2a2p and H_7do3ap, which react about ten times faster than H_8dotp and 10–100 times faster than H_4dota in the pH range 3–5. However, H_4dota and H_8dotp react similarly fast at pH 1–2. It is commonly accepted that macrocyclic ligands with coordinating pendant arms bind any metal ions in a two-step mechanism: formation of an *out-of-cage* complex, $[Cu(H_mL)]^*$, where mostly pendant arms are coordinated, followed by a rate-determining step, where the proton from the ring amine group(s) is transferred to the bulk solvent and the *in-cage* complex is simultaneously formed. This mechanism has also been suggested for the formation of Cu(II) complexes with H_3do3a[51] (Fig. 1) and H_4dota.[16] As the rate-determining step is ring amine deprotonation, it is useful to compare the reactivity with species with double protonated ring nitrogen atoms and with the same number of protonated pendant arms. This could be done as the $-PO_3H^-$ group can be stably coordinated to the central atom, similarly to the deprotonated carboxylic group. The reactivity of *e.g.* $(H_2dota)^{2-}$ and (*trans*-H_4do2a2p)$^{2-}$ species, which differ only in the protonation state of the phosphonates, is similar, unlike the reactivity of species with one additional protonated carboxylic group, *e.g.* $(H_3dota)^-$ and (*trans*-H_5do2a2p)$^-$. The reactivity is distinctly enhanced (≈3 orders of magnitude; Table 2) once a basic fully deprotonated phosphonate group is present, *e.g.* see the comparison of (*trans*-H_4do2a2p)$^{2-}$ with (*trans*-H_3do2a2p)$^{3-}$ or $(H_4doa3p)^{3-}$. Considering the most distant members of the ligand series, the $(H_4dotp)^{4-}$ species with two monoprotonated phosphonates

Table 1 Partial second-order formation rate constants, ${}^{f}k_{H_nL}$, for formation of the Cu(II) complexes (25 °C, I = 0.1 M (K,H)Cl). Protonation constants used in the calculations are given in Table S2. Data for Cu(II) complex with cyclen (an example of an amine ligand) are given for comparison

Rate constant, M^{-1} s^{-1}	Cyclen[a]	*trans*-H_2do2a	H_4dota	*trans*-H_6do2a2p	H_7doa3p	H_8dotp
${}^{f}k_{H_5L}$	—	—	—	50(3)	$1.60(1) \times 10^3$	$6.50(14) \times 10^4$
${}^{f}k_{H_4L}$	—	—	36.9(3)	$9.0(7) \times 10^3$	$3.45(9) \times 10^6$	$3.00(2) \times 10^6$
${}^{f}k_{H_3L}$	—	—	$7.64(20) \times 10^2$	$1.70(6) \times 10^7$	—	—
${}^{f}k_{H_2L}$	0.18–0.6	$3.2(7) \times 10^2$	$2.32(19) \times 10^4$	—	—	—
${}^{f}k_{HL}$	$(1.84–4.3) \times 10^6$	$1.47(2) \times 10^8$	$1.43(28) \times 10^9$	—	—	—
${}^{f}k_{H_4L}/{}^{f}k_{H_5L}$	—	—	—	180	2150	46
${}^{f}k_{H_3L}/{}^{f}k_{H_4L}$	—	—	20.7	1880	—	—
${}^{f}k_{H_2L}/{}^{f}k_{H_3L}$	—	—	30.3	—	—	—
${}^{f}k_{HL}/{}^{f}k_{H_2L}$	—	4.55×10^5	6.16×10^4	—	—	—

[a] Ref. 50 and 52.

Table 2 Times for full formation of the Cu(II) complexes at various pH (99% formation; $c_L = 10c_{Cu}$, c_{Cu} = 0.1 mM, c_L = 1 mM, 25 °C)

Acidity	*trans*-H_2do2a	H_4dota	*trans*-H_6do2a2p	H_7doa3p	H_8dotp
pH 6.0 (in ms)	110	15.5	0.98	1.86	3.80
pH 4.0 (in s)	12.3	1.02	0.046	0.0341	0.84
pH 2.0 (in min)	77.3	8.82	0.53	0.21	1.33

is as reactive as the $(H_2dota)^{2-}$ species with fully deprotonated pendant arms, despite the highest basicity of the ring amine groups in H_8dotp. This confirms again the importance of the strong interaction of fully deprotonated phosphonates with the metal ion in the supposed *out-of-cage* intermediate and/or the better ability of the phosphonates to transfer a proton from the ring amines to the bulk solution. Once the ring amines are only monoprotonated, the reactivity of such species is greatly increased, *e.g.* the (H*trans*-do2a)$^-$ or $(Hdota)^{3-}$ anions react much faster than the (H_2*trans*-do2a) or $(H_2dota)^{2-}$ species ($^fk_{HL}/^fk_{H_2L}$ = 455 000 and 61 600, respectively).

At most pH values in the studied pH range, complex formation is accelerated with the increasing number of phosphonic acid pendant arms (Table 2). This could be explained by the acid–base and coordination behaviour of the cyclic amines and phosphonic acid group. The phosphonic acid group is more acidic than the carboxylic group and, thus, phosphonates are able to bind metal ions in more acidic solutions. At lower pH, the complexation rate is less decreased for phosphonate-containing ligands. On the other hand, the fully deprotonated phosphonate group is more basic than the carboxylate group and interacts with metal ions more efficiently. Full deprotonation of the phosphonate group(s) also increases the ligand's overall negative charge, thus facilitating the ligand-metal ion electrostatic interactions. With an increasing number of phosphonic acid pendants, the stability of an intermediate *out-of-cage* complex is increased and this leads to a faster overall complex formation (Table 2). However, this trend could be offset by the rather high basicity of the ring amine groups as well as a too high stability of the intermediate *out-of-cage* complex, *e.g.* due to the presence too many well-complexing pendant arms, such as phosphonates. This probably becomes important during Cu(II) complexation with *e.g.* H_8dotp, as the complex is formed more slowly than the complexes with its analogues. In the diprotonated macrocyclic ligand species, the protons are shared among ring amines and the access of a metal ion into the ligand cavity is blocked. Once the first ring amine proton is removed, *in-cage* complex formation is greatly accelerated. As phosphonates are known to form rather strong hydrogen bonds, they efficiently assist proton removal from the ligand cavity. Here, the difference in reactivity between H_2L and HL species (with two or one proton(s) on the ring amine, respectively) of the phosphonate-containing ligands could not be investigated; however, the high reactivity of their even more protonated ligand species indirectly points to their ability to transfer the proton(s). A similar complexation rate enhancement has already been observed for the reaction of Cu(II) with methylphosphonic acid cyclam derivatives.[20] There are clear dependences of the partial rate constants on a number of phosphonic acid groups in the ligands or on the values of their first protonation constants (Fig. S20 and S21†).

Decomplexation kinetics

The decomplexation kinetics of the Cu(II) complexes was studied under acid catalysis in order to follow this process in a reasonable time. $NaClO_4$ was used as a background electrolyte as we have recently shown that nitrate anions, other commonly considered 'inert' anions, can interact with macrocyclic ligands and/or complexes.[23] Examples of data for the dissociation of Cu(II)–*trans*-H_6do2a2p/*trans*-H_2do2a complexes are shown in Fig. 3 (for more data, see Fig. S23–S27†).

As given above, some complex species protonated on the pendant arm(s) are thermodynamically stable and, thus, kinetically inactive. Therefore, additional proton(s) have to be bound to the complex species to promote their dissociation. On the basis of the kinetics data for the complexes of a number of macrocyclic ligands, the dissociation mechanism in Scheme 2 can be postulated, which leads to eqn (2):

$$k_{d,obs} = \frac{k_1 \times K \times [H^+] + k_2 \times K \times [H^+]^2}{1 + K \times [H^+]} = \frac{K \times [H^+](k_1 + k_2 \times [H^+])}{1 + K \times [H^+]} \quad (2)$$

where K is the protonation constant for formation of the first kinetically active species and k_1 and k_2 are rate constants char-

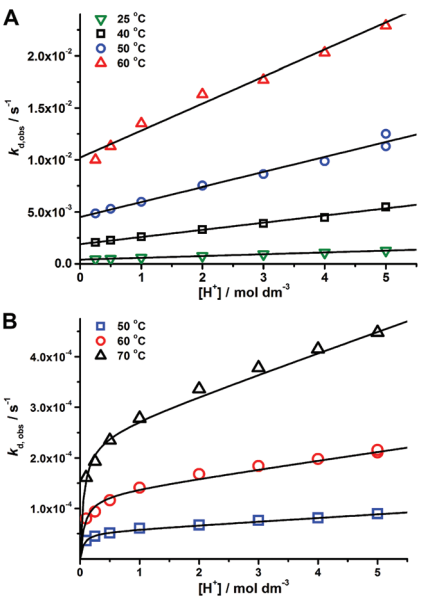
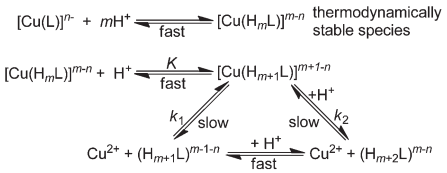


Fig. 3 Pseudo-first-order rate constants as a function of proton concentration for acid-assisted decomplexation of the [Cu(*trans*-do2a2p)]$^{4-}$ (A) and [Cu(*trans*-do2a)] (B) complexes (I = 5.0 M (Na,H)ClO_4).



Scheme 2 Proposed reaction scheme for acid-assisted decomplexation of the Cu(II) complexes. The charges of the ligand/complex species are omitted for clarity and a number of protons in the scheme (*m*) vary for different ligands.

acterizing the spontaneous (solvent assisted) and further proton-assisted (direct attack of a proton) decomplexation reactions, respectively. More detailed mechanisms for the decomplexation of Cu(II)-macrocycle complexes have been considered; however, not all the rate constants could be determined and the final rate law was analogous to that presented in eqn (2).[53] If the relation $1 \ll K\cdot[H^+]$ is valid, then the simplified eqn (3) is obtained:

$$k_{d,obs} = k_1 + k_2 \times [H^+] \quad (3)$$

Linear dependence (eqn (3)) was found for complexes of the octadentate ligands (H_4dota–H_8dotp), while eqn (2) had to be used for the *trans*-H_2do2a complex (Fig. 3). The protonation constant for formation of the reaction intermediate could be calculated only for the *trans*-H_2do2a complex. The complete set of results is shown in ESI (Table S5†). In addition, the dependences of the rate constants k_1 and k_2 on temperature were used to estimate the activation parameters for each reaction pathway, and the results are given in Table 3.

To compare the effect of an increasing number of phosphonate pendant arms in the ligand series on the kinetic inertness of their Cu(II) complexes, $^{d}k_{obs}$ *vs.* $[H^+]$ plots were compared (Fig. 4 and S28†). The most kinetically inert is the Cu(II)–H_4dota complex, while the inertness of the Cu(II) complexes is monotonously decreased with the higher number of phosphonic acid groups at any solution acidity. The constants k_1 and k_2, representing both reaction pathways, were 10^4- and 10^3 times higher for H_8dotp than for H_4dota, respectively (Fig. 5). Acceleration of the acid-assisted decomplexation of the Cu(II) complexes is more pronounced in the H_4dota–H_5do3ap–*trans*-H_6do2a2p series than for H_7doa3p/H_8dotp with more phosphonic acid pendants (Fig. 4 and 5). In addition, direct proton attack (k_2 pathway) is slower than the simple proton transfer pathway (k_1 pathway), and this difference is more significant for H_4dota than for H_8dotp (Table 3 and Fig. 5).

The k_1 and k_2 rate constant values could be correlated (Fig. S29 and S30†) with the ring amine basicity of the ligands ($\log K_2$) as well as with the overall protonation constants of the CuL complexes ($\log \beta_p$(CuL)) as these values represent the capability of the Cu(II) complexes to be protonated on the free pendant arms. Both dependences indirectly prove that proton transfer from protonated phosphonate group(s) to the ring

Table 3 Kinetic parameters for acid-assisted decomplexation of Cu(II) complexes of the studied ligands (t = 25 °C, I = 5.0 M (Na,H)ClO_4). For the complete set of results, see ESI (Table S5)

Parameter	Rate constant	$\Delta H^{\neq}$ kJ mol^{-1}	$\Delta S^{\neq}$ J mol^{-1} K^{-1}
H_4dota[a]			
$k_1/10^{-5}$, s^{-1}	*0.584*[b]	65	−128
$k_2/10^{-5}$, M^{-1} s^{-1}	*0.0206*[b]	107	−15
k_2/k_1, M^{-1}	*0.035*[b]		
$\tau_{1/2}$ (pH 2), h	**33.0**		
H_5do3ap[a]			
$k_1/10^{-5}$, s^{-1}	*7.84*[b]	66(1)	−101(4)
$k_2/10^{-5}$, M^{-1} s^{-1}	*0.43*[b]	105(2)	−3(5)
k_2/k_1, M^{-1}	*0.055*[b]		
$\tau_{1/2}$ (pH 2), h	**2.45**		
trans-H_6do2a2p			
$k_1/10^{-3}$ s^{-1}	0.411(8)	73(1)	−64(4)
$k_2/10^{-3}$ M^{-1} s^{-1}	0.173(3)	62(3)	−110(10)
k_2/k_1, M^{-1}	0.42(1)		
$\tau_{1/2}$ (pH 2), h	**0.47**		
H_7doa3p			
$k_1/10^{-3}$, s^{-1}	1.51(4)	80(2)	−32(8)
$k_2/10^{-3}$, M^{-1} s^{-1}	0.87(1)	74(5)	−55(16)
k_2/k_1, M^{-1}	0.58(2)		
$\tau_{1/2}$ (pH 2), min	**7.60**		
H_8dotp			
$k_1/10^{-3}$, s^{-1}	4.33(7)	72(3)	−50(9)
$k_2/10^{-3}$, M^{-1} s^{-1}	1.12(3)	75(5)	−50(16)
k_2/k_1, M^{-1}	0.26(1)		
$\tau_{1/2}$ (pH 2), min	**2.70**		
trans-H_2do2a			
$k_1/10^{-5}$, s^{-1}	*0.605*[b]	68(5)	−117(16)
$k_2/10^{-5}$, M^{-1} s^{-1}	*0.0563*[b]	78(2)	−102(6)
k_2/k_1, M^{-1}	*0.093*[b]		
log K	*1.35*[b]	−8	2
$\tau_{1/2}$ (pH 2), h	**31.5**		
H_3do3a[a]			
$k_1/10^{-5}$, s^{-1}	0.907	49	−178
$k_2/10^{-5}$, M^{-1} s^{-1}	0.168	73	−110
k_2/k_1, M^{-1}	0.093		
$\tau_{1/2}$ (pH 2), h	**21.2**		

[a] Taken from ref. 18 for ligand structures, see Fig. 1. [b] Extrapolated from the temperature dependence using the activation/thermodynamic parameters.

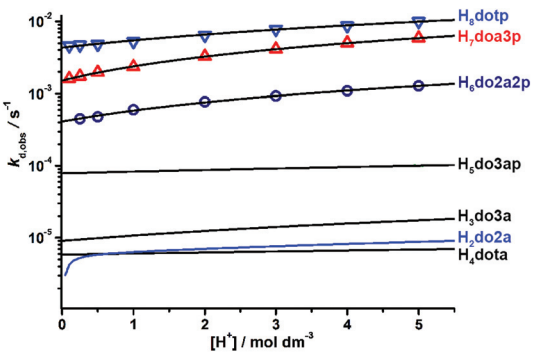


Fig. 4 Comparison of the decomplexation rate constants, $^{d}k_{obs}$, of the studied Cu(II) complexes (25 °C, I = 5.0 M (Na,H)ClO_4). Lines for H_3do3a, H_4dota and H_5do3ap are based on data from ref. 18.

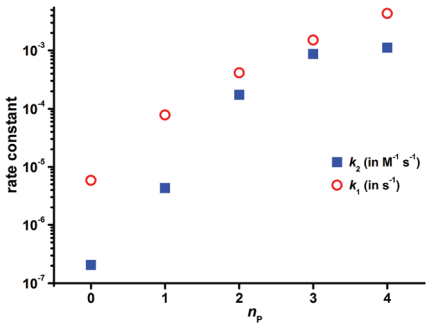


Fig. 5 Dependence of the rate constants, k_1 and k_2, for the acid-assisted dissociation of Cu(II) complexes on a number of phosphonic acid groups (n_P) in H_4dota and its analogues as obtained at 25 °C. The experimental data for H_4dota and H_5do3ap complexes were taken from ref. 18.

amines (k_1) is more important with an increased number of phosphonates, as well as that direct proton attack (k_2) can be mediated by the phosphonate pendants through the formation of hydrogen bonds between an arm oxygen atom and a ring nitrogen atom. Direct proton attack (k_2 pathway) seems to be more sensitive to the ligand and/or Cu(II) complex basicity than proton transfer (k_1 pathway). In addition, phosphonate groups are also able to interact with metal ions even in acidic solutions.[20a,46,54,55] Therefore, an increasing number of phosphonate groups assists better in removal of the metal ion from the ligand cavity. Isokinetic plots (Fig. S31†) demonstrate the analogous reaction mechanism for the k_1 pathway, while the trend for the k_2 pathway is different for the decomplexation of the Cu(II)–H_4dota and Cu(II)–H_5do3ap complexes. Overall, the kinetic inertness expressed as decomplexation half-times (Table 3) significantly decreases with the increasing number of phosphonate groups.

The H_4dota-like ligands have four pendant arms, but only one or two of them can be coordinated in the *in-cage* Cu(II) complexes. To investigate the influence of the presence of an "excess" of pendant arms, the dissociation kinetics of the Cu(II)–*trans*-H_2do2a complex (Fig. 3) was also studied. The high kinetic inertness ($\tau_{1/2} \approx$ 20–30 h at pH 2) is retained in the *trans*-H_2do2a–H_3do3a–H_4dota series, since both k_1 and k_2 parameters are almost the same. This highlights that the presence of non-coordinated carboxylates does not have an important effect on the acid-assisted decomplexation. On the other hand, the kinetic inertness decreases by one order or two orders of magnitude for the H_3do3a/H_5do3ap and *trans*-H_2do2a/-H_6do2a2p pairs, respectively, due to the presence of phosphonate groups.

There are some published dissociation kinetic data for Cu(II) complexes of other cyclen derivatives, *e.g.* the complex of *N*,*N*′,*N*″,*N*‴-tetrakis(2 hydroxoethyl)cyclen with weakly coordinating alcohol pendant arms is very kinetically inert,[56] while complexes of tetrakis(phosphinate) cyclen derivatives are similarly as labile as the Cu(II)–H_8dotp complex as the phosphinate groups are able to bind the metal ion in acidic solutions.[21,22]

Solid-state structure of KH_6doa3p·$3H_2O$

A single crystal of KH_6doa3p·$3H_2O$ was obtained by slow evaporation of the solvent from an acidic aqueous solution of H_7doa3p. The experimental and fitting details are given in Table S6.† The molecular structure of the ligand anion is shown in Fig. 6 and the crystal packing in Fig. S32,† while the most relevant bond lengths and angles of the ligand anion are presented in Table S7.†

In the structure of the $(H_6$doa3p$)^-$ anion, two ring nitrogen atoms are protonated, and each pendant arm is monoprotonated on its oxygen atom. The protonated oxygen atoms are clearly distinguished through the bond lengths (C=O 1.208 Å *vs.* C–OH 1.313 Å, and P–O 1.48–1.53 Å *vs.* P–OH 1.55–1.59 Å; Table S7†). The ring nitrogen atoms are almost coplanar and all the pendant arms are oriented to the same side of the plane. Two phosphonate pendant arms attached to the non-protonated nitrogen atoms are positioned just above the N_4-plane. They are connected by a strong hydrogen bond (O1–H···O7: *d*(D–A) 2.51 Å, ∠(D–H-A) 162°) and are involved in the intramolecular hydrogen bond network (Fig. 6 and Table S7†). One of the phosphonate groups forms a medium-strong hydrogen bond to a protonated ring amine adjacent over the ethylene chain (N10–H···O7: *d*(D–A) 2.93 Å, ∠(D–H-A) 145°) and this causes asymmetry in the network of hydrogen bonds between the ring amine groups (*d*(D–A) 2.84–3.9 Å). Such an arrangement has been found in zwitterionic H_8dotp,[57] where the ring nitrogen and pendant arm protonation scheme as well as connectivity are the same; thus, the intramolecular hydrogen bond parameters are analogous. Different arrangements were found in the zwitterionic forms of H_5do3ap[26] and *trans*-H_6do2a2p,[28] where the protonated nitrogen atom bears a monoprotonated phosphonate group, which is involved in an intramolecular hydrogen bond with this $>NH^+$ group, while the other pendants are directed outside. The other pendant arms of the $(H_6$doa3p$)^-$ anion are bound to the protonated nitrogen atoms and are oriented out of the macrocycle ring. All the pendant arms form a complex system of strong intermolecular hydrogen bonds (*d*(D–A) 2.47–2.68 Å, ∠(D–H-A) 166–174°; Fig. S32 and Table S7†). This leads to double-chains

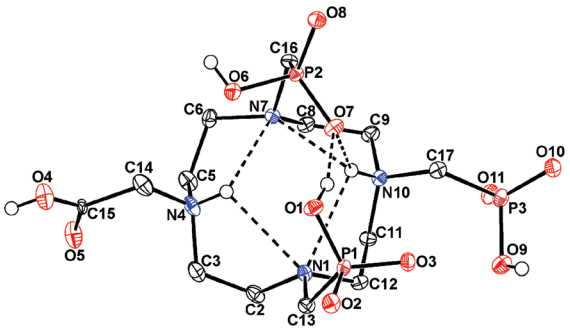


Fig. 6 Molecular structure of $(H_6$doa3p$)^-$ anion as found in the solid-state structure of KH_6doa3p·$3H_2O$. Intramolecular hydrogen bonds are shown as dashed lines.

of ligand molecules linked by the strong hydrogen bonds interconnected through K^+ cation layers.

Conclusions

We showed that the reactivity of H_4dota-like ligands towards the Cu(II) ion is significantly altered after substitution of the acetate pendant arms(s) by methylphosphonic acid one(s). This can be explained by the easier formation of an *out-of-cage* intermediate[24] due to the higher charge and high complexing ability of the deprotonated phosphonate groups and/or easier transfer of proton(s) from the ring amine group(s) to the bulk solvent assisted by the phosphonates. The rate of decomplexation of the Cu(II) complexes is also highly influenced by the presence of phosphonates since they probably affect the transfer of proton(s) from both the solvent and the already protonated pendant arms into the ligand cavity. In addition, the basicity of the amine groups is increased with the number of phosphonic acid pendant arms and, therefore, the amino groups are more prone to bind proton(s). Stability constants of the Cu(II)–H_8dotp system reported in the literature were re-evaluated. The stability constant of the $[Cu(dotp)]^{5-}$ complex is one the highest determined till now and is due to the very high ligand basicity and stable dinuclear complexes, where the additional Cu(II) ion is bound to non-coordinated phosphonate groups that are formed even in slightly acidic solutions.

The results in the present study can be utilized in the design of ligands for the complexation of copper radioisotopes. These and other published results indicate that the presence of phosphonic acid group(s) increases the Cu(II) complexation rates of both cyclen and cyclam derivatives. However, their influence on decomplexation rates is less clear. Here, the presence of phosphonic acid groups highly facilitates decomplexation. Unlike complexes of the title cyclen derivatives, Cu(II) complexes of the cyclam methylphosphonic acid derivatives (*e.g.* 1,8-H_4te2p; Fig. 1) with not fully substituted ring nitrogen atoms are kinetically inert, often even more so than the complexes of the analogous ligands with acetic acid pendants. However as shown here, substitution of the cyclen skeleton with methylphosphonic acids is not a suitable way to obtain good ligands for copper radioisotopes.

Acknowledgements

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