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Ternary Complexes of Zinc(II), Cyclen and Pyridinecarboxylic Acids

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Binary and ternary systems containing Zn^{2+}, cyclen and/or pyridinecarboxylic acids and amides [picolinic (HL^1), nicotinic (HL^2) and dipicolinic (H_2L^3) acids and nicotinamide (L^4)] have been investigated in solution and in the solid state. Dissociation and stability constants for the ligands and binary systems, respectively, are in accordance with literature results. Both picolinic and dipicolinic acids form stable ternary complexes with the $[Zn(cyclen)]^{2+}$ species; a weak interaction is observed for nicotinic acid and nicotinamide. The formation of ternary complexes stabilizes the products of protonation at the cyclen nitrogen atom, and these protonated complexes are found even up to pH = 7. The structures of the complexes in solution correlate well with those found in the solid state and by molecular modelling calculations. The solid-state structures of several complexes are determined. The zinc atom in the $[Zn(cyclen)(NO_3)]^+$ complex is bound in an N_4O tetragonal-pyramidal environment. The nicotinic acid anion forms a bridge in the $[\{Zn(cyclen)\}_2(\mu\text{-}L^2\text{-}N,O)]^{3+}$ cation, and both $[Zn(cyclen)]^{2+}$ units exhibit tetragonal-pyramidal arrangements. An unusual coordination mode is found in the $[\{Zn(cyclen)\}_2(\mu\text{-}L^1\text{-}O{:}N,O')]^{3+}$ cation, where the picolinic acid anion also bridges two $[Zn(cyclen)]^{2+}$ units but is coordinated monodentately through one oxygen atom to one unit (N_4O tetragonal pyramid) and chelates through the other pyridine nitrogen and carboxylate oxygen atoms. Nicotinamide in the $[Zn(cyclen)(L^4\text{-}N)]^{2+}$ cation is bound to the $[Zn(cyclen)]^{2+}$ unit through the pyridine nitrogen atom in the axial position of the tetragonal pyramid. This is the first fully quantitative assessment of Zn^{2+}-cyclen-ligand ternary systems, and the results, namely the presence of protonated cyclen in the ternary species even at high pH, are relevant for evaluation of the $[Zn(cyclen)]^{2+}$ unit as a model complex for zinc-containing enzymes.

Introduction

Zinc is an essential element for all forms of life and plays a critical role in various functions, both structural and catalytic, in proteins and enzymes.[1] Typically, the metal ion is coordinatively saturated by four donor atoms from amino acid side-chains of the protein when playing a structural role. On the other hand, if the functional catalytic role is examined, the ion in the active site is mostly three- or four-coordinate or, less commonly, the coordination number can also reach four, five and rarely even six, although this is always accompanied by coordination of labile ligands.[2] There are two basic mechanisms by which zinc(II) exerts its catalytic function. The first is exemplified by carbonic anhydrase (CA) and involves delivery of a metal-bound hydroxido nucleophile, which can exist at pH values where the concentration of free hydroxide ion is extremely low. The second takes advantage of the strong Lewis acid character of zinc(II) to polarize a susceptible bond for substitution,[3] and thus zinc(II) appears to be the most abundant metal centre present in phosphatases and nucleases.[4] In addition, the zinc enzymes remain a central issue in the area of molecular recognition.[5] For example, CA recognizes not only its normal substrates (CO_2 and HCO_3^-) but also substrates such as phosphoric esters, carboxylic esters, aldehydes and binds halide anions, SCN^-, acetate, sulfonamides, carboxamides, phenol, imidazole and alcohols as inhibitors. Up-to-date information regarding zinc(II) functions in biological systems has been collated recently.[6]

An important contribution to the detailed understanding of the catalytic activity and molecular recognition of the enzyme has been made by experimental investigations with suitable model systems. Several ligands, usually with three or four nitrogen donor atoms, have been used to mimic the rigid and stable coordination centre of the active zinc(II) part of an enzyme. Various azacycles, especially 1,4,7,10-tetraazacyclododecane (cyclen) and its derivatives, have been used to model the protein environment of carbonic anhydrase,[7,8] phosphatases,[9–11] β-lactamase,[12] flavoenzymes[13] or NADH models.[14] A flavin-$[Zn(cyclen)]^{2+}$ complex has also been used as a catalyst for the photooxidation

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of methoxybenzyl alcohol.[15] Cyclen ligands modified with other functionalities in the pendant arms, such as sulfonamides, carboxamides, phenols, alcohols or phosphoric esters, are also commonly used in these studies.[5]

There are only a few examples in the literature in which interactions or reactions of an unmodified $[Zn(cyclen)]^{2+}$ complex with another molecule have been studied. In addition to the examples of carbonic anhydrase mentioned above,[7,8] this complex has also been used to investigate the interaction and hydrolysis behaviour of phosphate esters[9,16] and has been found to catalyze the hydrolysis of *N*-benzylpenicillin.[17]

Most of the papers dealing with models based on $[Zn(cyclen)]^{2+}$ complexes are oriented towards the molecular recognition of nucleobases. These biomimetic studies, which have been summarised recently,[18,19] show that the $[Zn(cyclen)]^{2+}$ complex can be used as a novel receptor for imide-containing nucleobases such as thymine (T) and uracil (U) in aqueous solution and at physiological pH. Coordination of a nucleobase through a Zn^{2+}–N ("imide") bond is enforced by interaction of the "urea" carbonyl groups with the hydrogen atoms of the ring NH groups in the complementary positions. The dissociation constants, K_d, determined for ternary complexes of zinc(II), substituted cyclen and T or U are of the order of 10 μM, and for analogous systems containing bis($[Zn(cyclen)]^{2+}$) or tris($[Zn(cyclen)]^{2+}$) complexes (mononuclear complex units linked covalently through *p*-xylene or mesitylene spacers) with corresponding di- and trinucleotides (TpTp or TpTpTp) are even in a nanomolar range. In addition, bis- or tris($[Zn(cyclen)]^{2+}$) complexes connected through an imidazole group[20] and the formation of assembled three-dimensional supramolecules have also been studied.[21–25] Recently, a polymer containing $[Zn(cyclen)]^{2+}$ units was synthesised and investigated as a creatine receptor.[26]

As mentioned above, in contrast to the large number of papers dealing with modified cyclen complexes, only a few works have focused on the complexation and formation of ternary cyclen complexes,[7,8] which would be crucial for modelling the complexation at the active centre of the enzymes. Thus, in this paper we report a detailed investigation of the formation of ternary complexes of $[Zn(cyclen)]^{2+}$ and picolinic (HL^1), nicotinic (HL^2), and dipicolinic (H_2L^3) acids or nicotinamide (L^4) as the approaching ligands. This series helps to explain the complexing properties of the $[Zn(cyclen)]^{2+}$ species towards aromatic heterocycles and/or carboxylate coordination, their preference, and possible interactions of non-coordinated groups with the cyclen skeleton.

Results and Discussion

Preparation of the Complexes $[Zn(cyclen)(NO_3)]ClO_4$, $[\{Zn(cyclen)\}_2(\mu\text{-}L^2\text{-}N,O)](ClO_4)_3$, $[\{Zn(cyclen)\}_2(\mu\text{-}L^1\text{-}O:N,O')](ClO_4)_3\cdot H_2O$, $[Zn(cyclen)(L^4\text{-}N)](ClO_4)_2$ and Their X-ray Crystal Structures

To assess the coordination modes in the ternary complexes formed with Zn^{2+}, cyclen and pyridinecarboxylic acids or nicotinamide in solution we tried to prepare single crystals suitable for X-ray analysis by slow evaporation of the solvent from mixed solutions of Zn^{2+}, cyclen and the corresponding pyridine-based ligand in an equimolar ratio at a concentration of about 0.1–0.2 M at various pH values. We succeeded in several cases, and the crystal structures of four crystalline materials were determined. The bond lengths found in the coordination spheres of the zinc(II) complexes and the deviations from planarity observed for the cyclen nitrogen atoms are given in Table 1. Further bond lengths and angles are listed in Table S1 (see Supporting Information).

Table 1. Selected bond lengths [Å] found in the crystal structures.

$[Zn(cyclen)(NO_3)]ClO_4$				$[Zn(cyclen)(L^4\text{-}N)](ClO_4)_2$			
Coordination sphere		Distance from N_4 plane		Coordination sphere		Distance from N_4 plane	
Zn–O1	1.998(3)	N11, N17	–0.037(2)	Zn–N1	2.029(3)	N11, N17	–0.005(2)
Zn–N11	2.097(4)	N14, N110	0.037(2)	Zn–N11	2.112(3)	N14, N110	0.005(2)
Zn–N14	2.148(5)	Zn	0.786(2)	Zn–N14	2.139(4)	Zn	0.786(2)
Zn–N17	2.124(4)			Zn–N17	2.136(3)		
Zn–N110	2.124(5)			Zn–N110	2.164(3)		
$[\{Zn(cyclen)\}_2(\mu\text{-}L^2\text{-}N,O)](ClO_4)_3$				$[\{Zn(cyclen)\}_2(\mu\text{-}L^1\text{-}O:N,O')](ClO_4)_3\cdot H_2O$			
Coordination sphere		Distance from N_4 plane		Coordination sphere		Distance from N_4 plane	
Zn1–N1	2.026(2)	N11, N17	–0.0075(13)	Zn2–O2	1.945(8)	N21, N27	–0.006(9)
Zn1–N11	2.123(2)	N14, N110	0.0075(13)	Zn2–N21	2.079(16)	N24, N210	0.006(9)
Zn1–N14	2.129(3)	Zn1	0.7849(13)	Zn2–N24	2.157(18)	Zn2	0.805(9)
Zn1–N17	2.146(2)	N21	–0.0069(14)	Zn2–N27	2.110(15)	Distance from O1,N11,N17,N1,Zn1 plane	
Zn1–N110	2.139(2)	N24	0.0068(14)	Zn2–N210	2.127(17)	O1	–0.084(6)
Zn2–O1	1.924(2)	N27	–0.0068(13)	Zn1–N1	2.154(9)	N1	0.059(6)
Zn2–N21	2.137(2)	N210	0.0069(14)	Zn1–O1	2.232(8)	N11	0.051(5)
Zn2–N24	2.133(3)	Zn2	0.7880(14)	Zn1–N11	2.128(10)	N17	–0.064(5)
Zn2–N27	2.168(3)			Zn1–N14	2.143(12)	Zn1	0.038(5)
Zn2–N210	2.111(2)			Zn1–N17	2.201(11)	N14	2.091(14)
				Zn1–N110	2.142(11)	N110	–2.055(13)

First, we obtained a single crystal of the parent Zn^{2+}-cyclen complex. $[Zn(cyclen)(NO_3)]ClO_4$ contains a five-coordinate Zn^{2+} ion (Figure 1) with four nitrogen atoms of the macrocycle and one oxygen atom of nitrate ion coordinated to the metal centre to form a slightly distorted tetragonal pyramid (τ = 0.097; this parameter should be 1.000 for an ideal trigonal bipyramid and 0.000 for an ideal tetragonal pyramid[27]). The zinc atom is placed above the plane containing the four nitrogen atoms of cyclen (0.750 Å). All four five-membered chelate rings of cyclen have the same conformation (δδδδ in the independent unit), as is usual in similar complexes. The bond lengths and angles around the Zn^{2+} ion (Tables 1 and S1) are as expected.

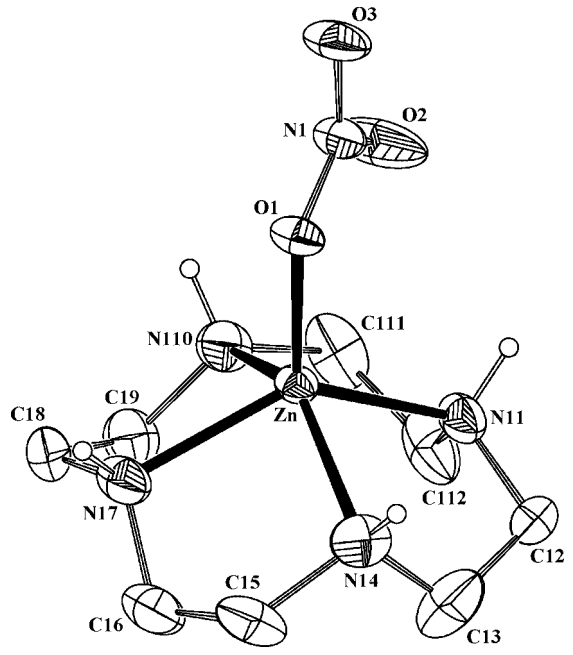


Figure 1. ORTEP drawing of the $[Zn(cyclen)(NO_3)]^+$ cation found in the structure of $[Zn(cyclen)(NO_3)](ClO_4)$. Hydrogen atoms bound to carbon atoms have been omitted for clarity.

$[\{Zn(cyclen)\}_2(\mu\text{-}L^2\text{-}N,O)](ClO_4)_3$ contains two $[Zn(cyclen)]^{2+}$ complex subunits bridged by nicotinate (Figure 2). All five-membered chelate rings of both cyclen ligands show the same conformation (λλλλ for both subunits in the independent unit). The fifth coordination sites are occupied by a pyridine nitrogen atom (N1–Zn1) and a carboxylate oxygen atom (O1–Zn2) to form slightly distorted tetragonal pyramids [the τ values for the coordination polyhedrons $Zn1(N_5)$ and $Zn2(N_4O)$ are 0.015 and 0.002, respectively]. The planar pyridine ring of the nicotinate anion is perpendicular to the cyclen plane formed by N11, N14, N17 and N110. The distances of Zn1 and Zn2 from the tetragonal basal planes formed by the nitrogen atoms of cyclen are 0.793 and 0.781 Å, respectively; these values are slightly longer than that observed in $[Zn(cyclen)(NO_3)]ClO_4$. The apical Zn1–N1 and Zn2–O1 bond lengths (2.026 and 1.924 Å, respectively) are shorter than the average Zn–N length (2.135 Å) in the basal plane of the cyclen units. Nevertheless, the bond lengths fall into the expected range for such complexes (Tables 1 and S1).[28]

$[\{Zn(cyclen)\}_2(\mu\text{-}L^1\text{-}O{:}N,O')](ClO_4)_3{\cdot}H_2O$ also contains two independent zinc atoms with two different coordination geometries, namely a distorted tetragonal bipyramid $[Zn1(N_5O)$ coordination sphere] and a slightly distorted tetragonal pyramid [$Zn2(N_4O)$ coordination sphere with a τ value of 0.002; Figure 3]. The distance of Zn2 from the tetragonal basal plane formed by the nitrogen atoms of the cyclen ring (0.790 Å) is very close to that found in the previous structure; the picolinate oxygen atom O2 is coordinated in the apical position. The central Zn1 atom is surrounded by four nitrogen atoms of cyclen, and the coordination sphere is completed by *cis* coordination of the picolinate nitrogen atom N1 and oxygen atom O1. The atoms form an axially elongated tetragonal bipyramid, with the

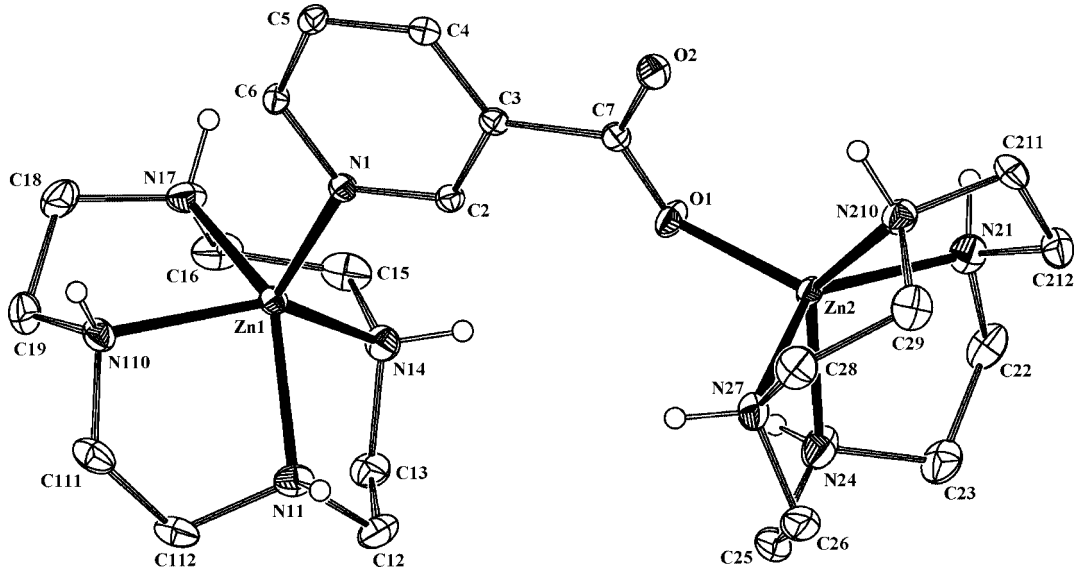


Figure 2. ORTEP drawing of the $[\{Zn(cyclen)\}_2(\mu\text{-}L^2\text{-}N,O)]^{3+}$ cation found in the structure of $[\{Zn(cyclen)\}_2(\mu\text{-}L^2\text{-}N,O)](ClO_4)_3$. Hydrogen atoms bound to carbon atoms have been omitted for clarity.

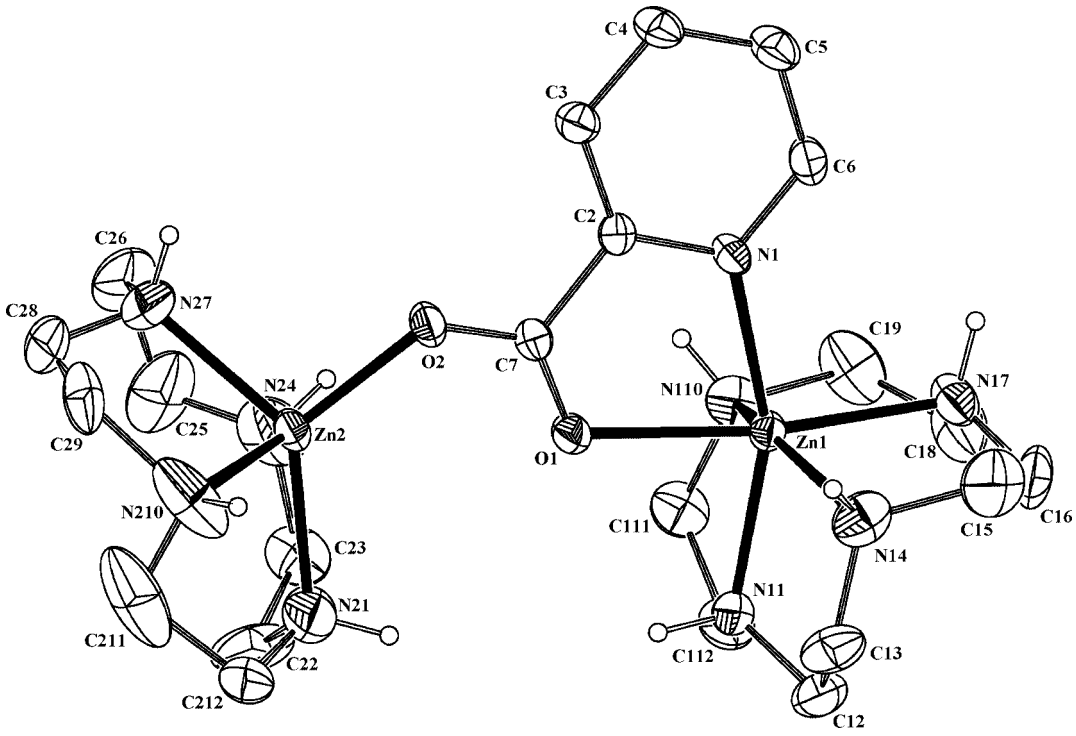


Figure 3. ORTEP drawing of the $[\{Zn(cyclen)\}_2(\mu\text{-}L^1\text{-}O{:}N,O')]^{3+}$ cation found in the structure of $[\{Zn(cyclen)\}_2(\mu\text{-}L^1\text{-}O{:}N,O')](ClO_4)_3\cdot H_2O$. Hydrogen atoms bound to carbon atoms have been omitted for clarity.

equatorial plane formed by three cyclen nitrogen atoms N11, N17 and N110 together with O1 from picolinate. However, the bond angles show large deviations from the corresponding ideal values. The coordination spheres of the Zn1 and Zn2 atoms contain different conformations of the cyclen. In the Zn2 sphere a λ conformation is observed for all four five-membered chelate rings formed due to coordination of the nitrogen atoms, whereas the nitrogen atoms of cyclen coordinated to Zn1 do not form a plane as in the other structures and the chelate rings adopt δλδλ conformations (see Figure S2 in the Supporting Information). Selected geometric parameters for this complex are listed in Table 1.

$[Zn(cyclen)(L^4\text{-}N)](ClO_4)_2$ contains monomeric units with a five-coordinate zinc atom (Figure 4). Four nitrogen atoms of the macrocycle and the nitrogen atom of nicotinamide are coordinated to the zinc atom in an approximately tetragonal-pyramidal sphere ($\tau = 0.017$). All four five-membered chelate rings of cyclen have the same conformation (λλλλ in the independent unit). Four cyclen nitrogen atoms N11, N14, N17 and N110 form a plane with a similar distance to the zinc atom as found in the previous structures (0.794 Å). The pyridine ring of nicotinamide is perpendicular to this plane. Selected geometric parameters are listed in Tables 1 and S1.

Two structural motifs are observed in the compounds studied. Thus, if the approaching ligand is monodentately coordinated to the $[Zn(cyclen)]^{2+}$ unit, the central Zn^{2+} ion is placed above the plane formed by the four nitrogen atoms of cyclen and the conformation of all four five-membered chelate rings is the same, either all-δ or all-λ. The distance

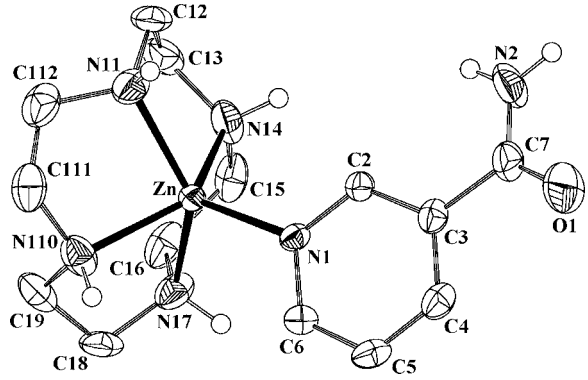


Figure 4. ORTEP drawing of the $[Zn(cyclen)(L^4\text{-}N)]^{2+}$ cation found in the structure of $[Zn(cyclen)(L^4\text{-}N)](ClO_4)_2$. Hydrogen atoms bound to carbon atoms have been omitted for clarity.

from the N_4 plane is 0.75 Å for $[Zn(cyclen)(NO_3)]ClO_4$ and about 0.79 Å for the ternary complexes, which means that it falls into the range 0.75–0.80 Å found in the CCDC[28,29] for Zn^{2+} complexes of cyclen derivatives with no coordinated pendant. If the pendant group of cyclen is coordinated, however, the N_4–Zn distance is usually longer. Formation of an N_4 plane containing the cyclen nitrogen atoms has also been observed in structures with higher coordination numbers, such as in the Zn^{2+} complexes with *N*,*N*′,*N*′′,*N*′′′-tetrakis(carbamoylmethyl)cyclen (dotam),[30] which has a coordination number of six, or with *N*,*N*′,*N*′′-tris(pyrazol-1-ylmethyl)cyclen,[31] which has a coordination number of seven. When the approaching ligand is bidentately coordinated to form a chelate, as found in

$[\{Zn(cyclen)\}_2(\mu\text{-}L^1\text{-}O{:}N,O')](ClO_4)_3\cdot H_2O$ (the coordination number of Zn^{2+} is six), this results in a different δλδλ conformation of cyclen. This conformation is relatively rare among Zn^{2+}-cyclen complexes and is found, for example, in $[Zn(H_2dota)]$ (H_4dota = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid)[32] and $[Zn(cyclen\text{-}R\text{-}O)(H_2O)]^{2+}$ (R = $CH_2COC_6H_4Br$-4).[33]

Pyridinecarboxylic acids also show a tendency to bridge the $[Zn(cyclen)]^{2+}$ units in dinuclear complexes. A similar bridging motif through another ligand has also been found for carbonate,[34] phosphate[35] and imidazole.[20] Crystallisation of the bridged dinuclear complexes from equimolar solutions is surprising but is probably due to their lower solubility due to the relatively hydrophobic surrounding sphere formed by the cyclen ligands.

Potentiometric Study

To prevent misunderstandings and to clarify the following text, both the protonation and stability constants are concentration constants and are defined by the general term:

$$\beta_{pqrs} = [H_p(cyclen)_q(L^n)_rZn_s]/[H]^p \times [cyclen]^q \times [L^n]^r \times [Zn]^s$$

This definition will be used for proton-ligand speciation and for all binary systems (Zn^{2+}-cyclen and Zn^{2+}-H_xL^n) as well as for the ternary systems. The relevant stoichiometric coefficient for the missing component is taken to be zero. In this formalism, each species can be represented by the four-digit code *pqrs*; for example, the $[Zn(cyclen)]^{2+}$ complex can be viewed as 0101, and $[Zn(Hcyclen)(HL^1)]^{2+}$ as 2111. The charges are omitted from the general formulae for the sake of clarity.

Protonation Constants of HL^1, HL^2, H_2L^3 and L^4 and Their Stability Constants with Zn^{2+} Ions

Although the protonation constants of pyridinecarboxylic acids and nicotinamide have already been reported and summarised,[36] we determined them under our experimental conditions (25 °C and I = 0.1 M KNO_3) by potentiometric titration. All values are listed in Table 2 together with the literature data for comparison. The $\log\beta_{1010}$ values correspond to the protonation of nitrogen atoms of the pyridine ring and the difference $\log\beta_{2010} - \log\beta_{1010}$ to the dissociation ($\log K_A$) of a proton from the carboxylic group.[36] In addition to the published protonation/dissociation constants, we were able to determine the $\log\beta_{3010}$ value for dipicolinic acid associated with protonation of the second carboxylate group.

The pyridine derivatives form complexes with a Zn^{2+}/H_xL^n molar ratio of 1:1 and 1:2 in the presence of Zn^{2+}; HL^1 also forms a complex with a 1:3 ratio. The species determined for each system together with their stability constants and literature values are listed in Table 2; representative distribution diagrams are shown in Figure S3 (see the Supporting Information). It is clear that there are no significant differences between our data and the literature data for the systems with picolinic and dipicolinic acids.[36] The slight differences found for the 1:2 and 1:3 ratios are probably caused by variants in ionic strength and electrolyte. In addition, these values are a little higher than those expected for these ratios from the values determined for the 1:1 (or 1:2) species, although they still lie in the expected range of 3σ variance. The formation of a zinc(II) hydroxide precipitate was observed at pH > 8.0 in both systems. Both picolinic and dipicolinic acids form chelates through the nitrogen atom and the carboxylate group (see below) in the pH range 2–7, which means that these complexes are more stable than those found for nicotinic acid and nicotinamide. Nicotinic acid forms very weak complexes with a low abundance in aqueous solution, and in acid solution the main species seems to be $[Zn(HL^2)]^{2+}$, probably with a protonated pyridine nitrogen atom and coordinated through the carboxylate group and, in the neutral region, $[Zn(L^2)]^+$ species with a coordinated nitrogen atom. The abundance of these species is only about 20% (Figure S3). The uppermost estimate of $\log\beta_{0011}$ for the $[Zn(L^4)]^{2+}$ complex is one, which corresponds to the value found in the literature.[36]

Protonation Constants of Cyclen and Stability Constants of Its Zn^{2+} Complexes

The protonation constants of cyclen are given in Table 3. From the best fits of our experimental data, it appears that

Table 3. Protonation constants of cyclen and stability constants of Zn^{2+}-cyclen complexes (25 °C, I = 0.1 M KNO_3).[a]

$\log\beta_{1100}$	$\log\beta_{2100}$	$\log\beta_{3100}$	$\log\beta_{4100}$	$\log\beta_{0101}$	$\log\beta_{1101}$
10.67(1)	20.25(2)	–	22.59(6)	14.77(2)	7.36(5)
10.65	*20.29*	*21.69*	*22.39*	*16.2*	*7.75*

[a] Values in italics are taken from ref.[36]

Table 2. Protonation constants of pyridinecarboxylic acids and nicotinamide and the stability constants of their Zn^{2+} complexes (25 °C, I = 0.1 M KNO_3).[a]

Ligand	$\log\beta_{1010}$	$\log\beta_{2010}$	$\log\beta_{3010}$	$\log\beta_{1011}$	$\log\beta_{0011}$	$\log\beta_{1011}$	$\log\beta_{2011}$	$\log\beta_{1021}$	$\log\beta_{0021}$	$\log\beta_{0031}$
HL^1	5.22(1)	6.39(1)	–	–	5.33(2)	–2.56(2)	–12.37(3)	–	9.60(7)	14.06(7)
	5.21	*6.16*			*5.23*				*9.56*	*12.9*
HL^2	4.66(1)	6.77(1)	–	6.18(3)	1.72(4)	–	–	–	–	–
	4.69	*6.77*								
H_2L^3	4.70(2)	6.72(3)	8.08(4)	–	6.50(8)	–	–	14.98(10)	13.18(6)	–
	4.66	*6.73*			*6.35*				*11.88*	
L^4	3.26(2)	–	–	–	<1	–	–	–	–	–
	3.31				*0.78*				*1.15*	

[a] Values in italics are taken from ref.[36]

Table 4. Stability constants of ternary Zn^{2+}-cyclen-H_xLn complexes (25 °C, I = 0.1 M KNO_3).

Ternary system	$\log\beta_{0111}$	$\log\beta_{1111}$	$\log\beta_{2111}$	$pK_A(1111)$	$pK_A(2111)$	$\log\beta_{0111} - \log\beta_{0101}$[a]
Zn-cyclen-HL^1	18.68(6)	24.09(7)	27.63(12)	6.22	3.53	3.91
Zn-cyclen-HL^2	16.72(18)	21.95(7)	25.62(17)	5.22	3.68	1.95
Zn-cyclen-H_2L^3	19.20(11)	26.09(11)	29.62(13)	6.89	3.53	4.43
Zn-cyclen-L^4	15.5(6)	20.3(2)	–	4.8	–	0.7

[a] Equilibrium constant for the reaction: $[Zn(cyclen)]^{2+} + (L^n)^{x-} \rightleftharpoons [Zn(cyclen)(L^n)]^{(2-x)+}$.

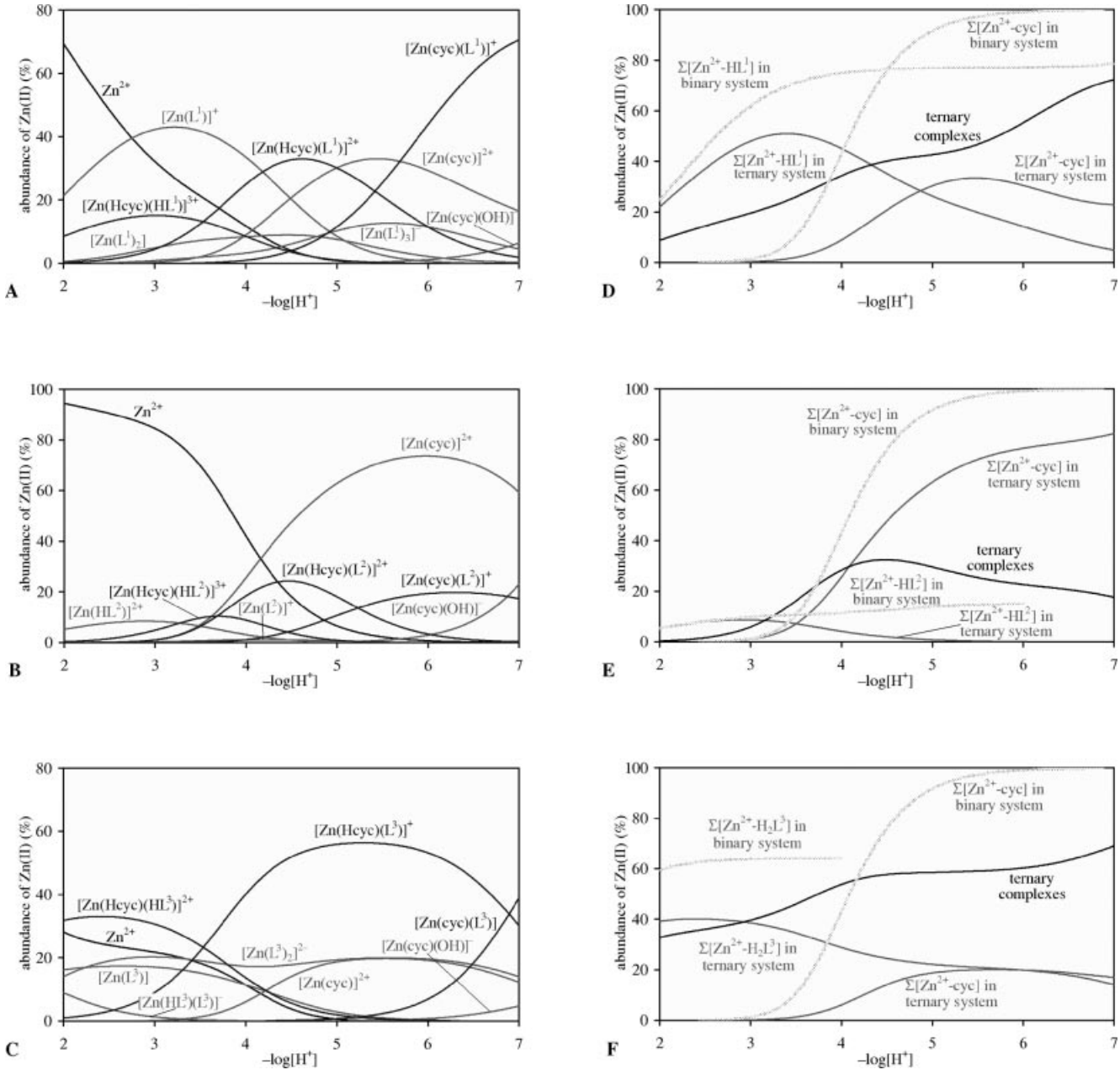


Figure 5. Calculated distribution of Zn^{2+} in the ternary Zn^{2+}-cyclen-H_xL^n systems as a function of –log[H^+] for a Zn^{2+}/cyclen/H_xL^n molar ratio of 1:1:1: (A) HL^1, (B) HL^2, (C) H_2L^3 [binary Zn^{2+}-cyclen species and binary Zn^{2+}-H_xL^n species (light grey) and ternary Zn^{2+}-cyclen-H_xL^n species (dark grey)]. A comparison of the total abundance of all binary Zn^{2+}-cyclen species and all binary Zn^{2+}-H_xL^n species in the ternary 1:1:1 mixtures (grey) and all ternary complexes (black) with total abundance of Zn^{2+}-cyclen and Zn^{2+}-H_xL^n (dotted) complexes found in the binary mixtures for Zn^{2+}/cyclen = 1:1 and Zn^{2+}/H_xL^n = 1:1: (D) HL^1, (E) HL^2, (F) H_2L^3. cyc = cyclen; $c(Zn^{2+})$ = 0.004 M in all cases.

binding of the third and fourth protons to the cyclen molecule occurs simultaneously. However, the value of $\log\beta_{4100} - \log\beta_{2100}$ (2.34) corresponds well with the sum of the pK_A values (1.4 + 0.7 = 2.1) reported in the literature.[36] The out-of-cell method had to be used for potentiometric titrations of the Zn^{2+}-cyclen system due to the slow rate of the complex formation. The dominant species is $[Zn(cyclen)(H_2O)]^{2+}$, whose deprotonation starts at pH > 6.0, and the hydroxido complex $[Zn(cyclen)(OH)]^{+}$ is the main species in solution at pH = 8. The determined stability constants together with the literature values are also listed in Table 3 and the distribution diagram is shown in Figure S4 (see the Supporting Information).

Stability Constants in Ternary Systems

The potentiometric titrations were performed by the out-of-cell method, as described in the Experimental Section. The determined stability constants are listed in Table 4 and the calculated distribution diagrams are shown in Figure 5A–C (except for the nicotinamide system, where the very low complex stabilities result in a very low abundance of the complex species). The distribution diagrams indicate the presence of binary $[Zn(L^n)]$ and $[Zn(cyclen)]^{2+}$ species. The abundance of the binary $[Zn(L^n)]$ species corresponds to their stability constant values, which are higher for the chelating ligands (picolinic and dipicolinic acids) than for nicotinic acid. The distribution of Zn^{2+} between the binary and ternary species is evident from Figure 5D–F. The total abundance of ternary $[H_{0-2}(cyclen)(L^n)Zn]$ complexes in the systems with picolinic and dipicolinic acids increases with pH up to 70% in the neutral region as the abundance of the binary species decreases. The total abundance of $[H_{0-2}(cyclen)(L^2)Zn]$ in the nicotinic acid system is only 30%. The distributions in the binary systems are also included in Figure 5D–F for comparison. The nicotinamide system is different, and only the values of stability constants for the monoprotonated and non-protonated complexes were estimated here. Its value is close to the lower limit of the used method, so the interaction between $[Zn(cyclen)(H_2O)]^{2+}$ and nicotinamide is very weak, if any.

Formation of the 2111, 1111 and 0111 ternary complexes was observed in the systems with picolinic, dipicolinic and nicotinic acids. The acidic and basic groups of both ligands offer several possible protonation schemes and their corresponding dissociation constants. Comparison of the pK_A values determined for the ternary systems with those found for the acids indicates the following scheme: formation of the diprotonated 2111 species starts at pH ≈ 2. These species deprotonate to the 1111 species with a $pK_A(2111)$ of about 3.5. These values are higher than the pK_A values of carboxylic acid groups but lower than those corresponding to the pyridine nitrogen atoms. The proton is released from the pyridine nitrogen atom of the diprotonated 2111 species, and the dissociation constants decrease (compared with the free ligands), probably due to simultaneous coordination to Zn^{2+}. However, $pK_A(1111)$ is even higher than pK_A of the protonated nitrogen atom of the pyridine ligands themselves (5–7), and thus this proton should be released from the cyclen amino group. The monoprotonated species can therefore be viewed as $[Zn(Hcyclen)(L^n)]$ and the diprotonated species as $[Zn(Hcyclen)(HL^n)]$; other microspecies with protonated carboxylic and pyridine functions could also be present.

The stability constants of the $[Zn(Hcyclen)(HL^n)]$, $[Zn(Hcyclen)(L^n)]$ and $[Zn(cyclen)(L^n)]$ species decrease in the order dipicolinic > picolinic > nicotinic acids (Table 4), in agreement with the stability constants of the binary systems (Table 2). The contribution of the acids to the stability of the ternary complexes {expressed as the equilibrium constant for the reaction $[Zn(cyclen)]^{2+} + (L^n)^{x-} \rightleftharpoons [Zn(cyclen)(L^n)]^{(2-x)+}$; $\log\beta_{0111} - \log\beta_{0101}$} is obviously lower than that of the $[Zn(L^n)]$ species for picolinic and dipicolinic acids. As these constants have a similar value to that corresponding to the formation of the $[Zn(cyclen)(imidazole)]^{2+}$ species ($\log K$ = 3.0),[37] which contains the more basic imidazole as a co-ligand, an extra stabilisation due to formation of the chelate ring should be assumed. These values are low (at the limit of potentiometry) in the case of nicotinic acid and nicotinamide and are therefore roughly comparable (see Table 4).

A structural motif in which the pyridinecarboxylic acid bridges two $[Zn(cyclen)]^{2+}$ subunits is observed in the complexes isolated in the solid state. All attempts to include a species of the $[\{Zn(cyclen)\}_2L^n]$ type into the chemical model to fit the potentiometric data failed. Thus, the presence of these species in solution is expected to be negligible; they crystallise in preference because of their low solubility.

^{1}H NMR Study

To confirm the complexation behaviour suggested in the potentiometric section above, we monitored the changes in the ^{1}H NMR spectra of a cyclen solution and its mixtures with Zn^{2+}. In the NMR spectra of cyclen itself, one peak of the CH_2 groups changes its position from δ = 3.03 ppm at pH = 1.9 to δ = 2.98 (pH = 9.0) and 2.66 ppm (pH = 12.2). The observed changes with pH correspond to the determined protonation constants.

A peak with a chemical shift close to that of free cyclen is observed for the system with Zn^{2+} at pH ≈ 2, therefore no complexation is expected (Figure 6A). At pH > 2.55, a set of peaks attributable to the $[Zn(cyclen)(H_2O)]^{2+}$ species, namely two multiplets at δ = 2.65–2.87 ppm (CH_2 groups) and a broad singlet at δ = 3.67 ppm (NH groups), appear in addition to the signal for free cyclen. One can therefore conclude that the complex formed is rigid and that free and complexed ligand do not mutually exchange on the NMR timescale. The signal attributed to free cyclen disappears at pH > 5.0, which corresponds to full complexation of Zn^{2+}. The NMR spectra also indicate deprotonation of the coordinated water. The formation of the $[Zn(cyclen)(OH)]^{+}$ hydroxido complex does not influence the multiplets of the CH_2 groups, although the position of the signal corresponding to the NH proton is shifted from $\delta \approx 3.6$ ppm at pH = 6.99 to $\delta \approx 3.3$ ppm at pH = 8.97. The changes ob-

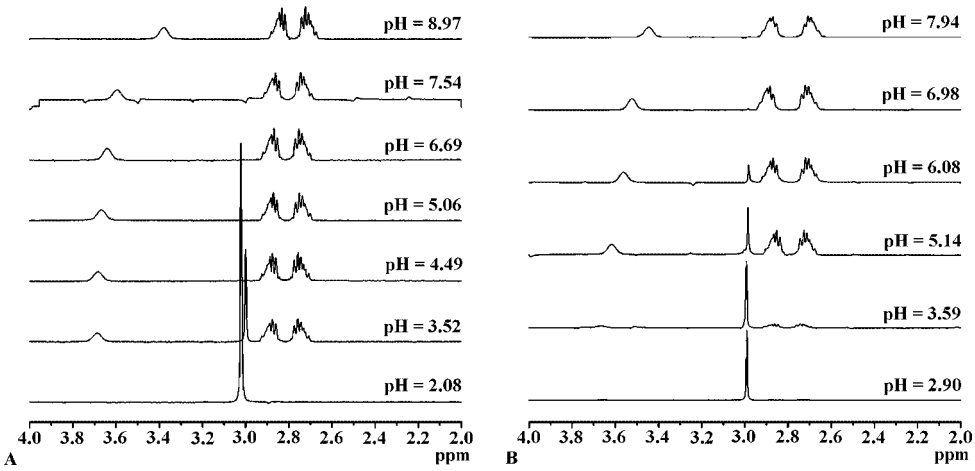
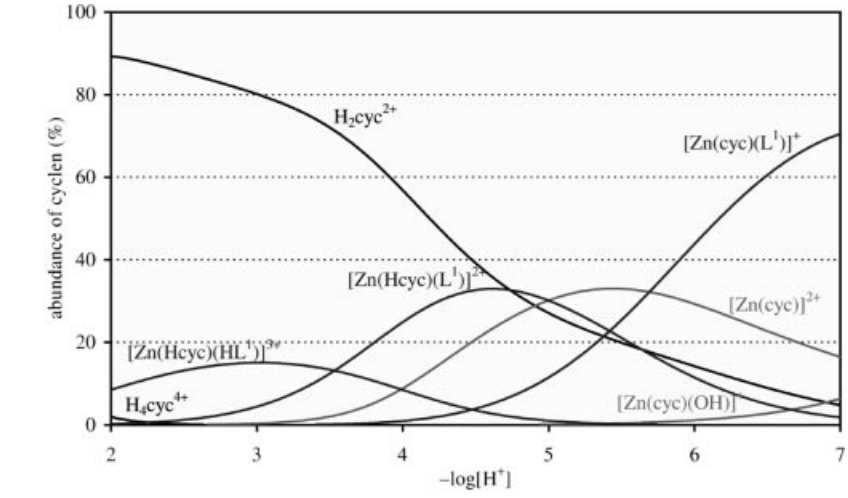
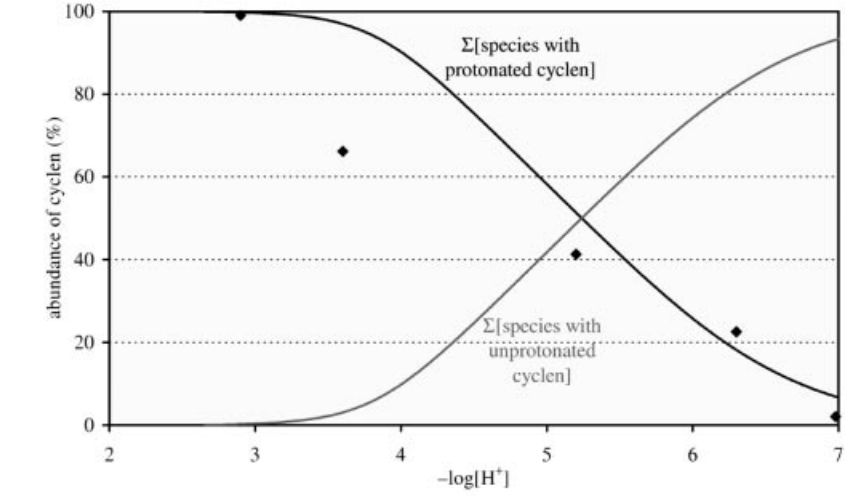


Figure 6. Examples of the ^{1}H NMR spectra of the studied systems at different pH values: (A) Zn^{2+}-cyclen (1:1 mixture); (B) Zn^{2+}-cyclen-HL^1 (1:1:1 mixture).



A



B

Figure 7. (A) Abundance of cyclen-containing species in the Zn^{2+}/cyclen/HL^1 (1:1:1) system; $c(Zn^{2+})$ = 0.004 M (ternary species: dark grey; binary species: light grey). (B) Comparison of the sum of the ^{1}H NMR signal integrals of protonated cyclen species (diamonds) and the total abundance of protonated cyclam species (black line) and the deprotonated $[Zn(cyclen)]^{2+}$ complex (grey line), as calculated by potentiometry.

served in the NMR spectra correspond well to the speciation determined by potentiometry.

The changes in the cyclen region of the ^{1}H NMR spectra of the ternary complexes as the pH is varied are also shown in Figure 6B for the system with picolinic acid. The trends in the spectra of the other ternary systems are similar. As is the case with the binary $[Zn(cyclen)]^{2+}$ species, the multiplets found close to δ = 2.8 ppm for all ternary systems can be assigned to deprotonated species of the type [Zn(cyclen)(L^n)]. On the other hand, a singlet close to δ = 3 ppm is found in the ternary systems and is present even up to pH ≈ 6 for picolinic acid and 7 for dipicolinic acid; its position is slightly shifted at pH ≈ 5 from δ = 3.02 (free cyclen) to 2.95 ppm. Distribution diagrams B and E in Figure 6 show that about 80% of cyclen should be taken up in the complexes and that the protonated species [Zn(Hcyclen)(HL^n)] and [Zn(Hcyclen)(L^n)] should also be present at this pH along with the [Zn(cyclen)(L^n)] species mentioned above. In contrast to [Zn(cyclen)(L^n)], however, these protonated species are not likely to be rigid. Thus, the signal

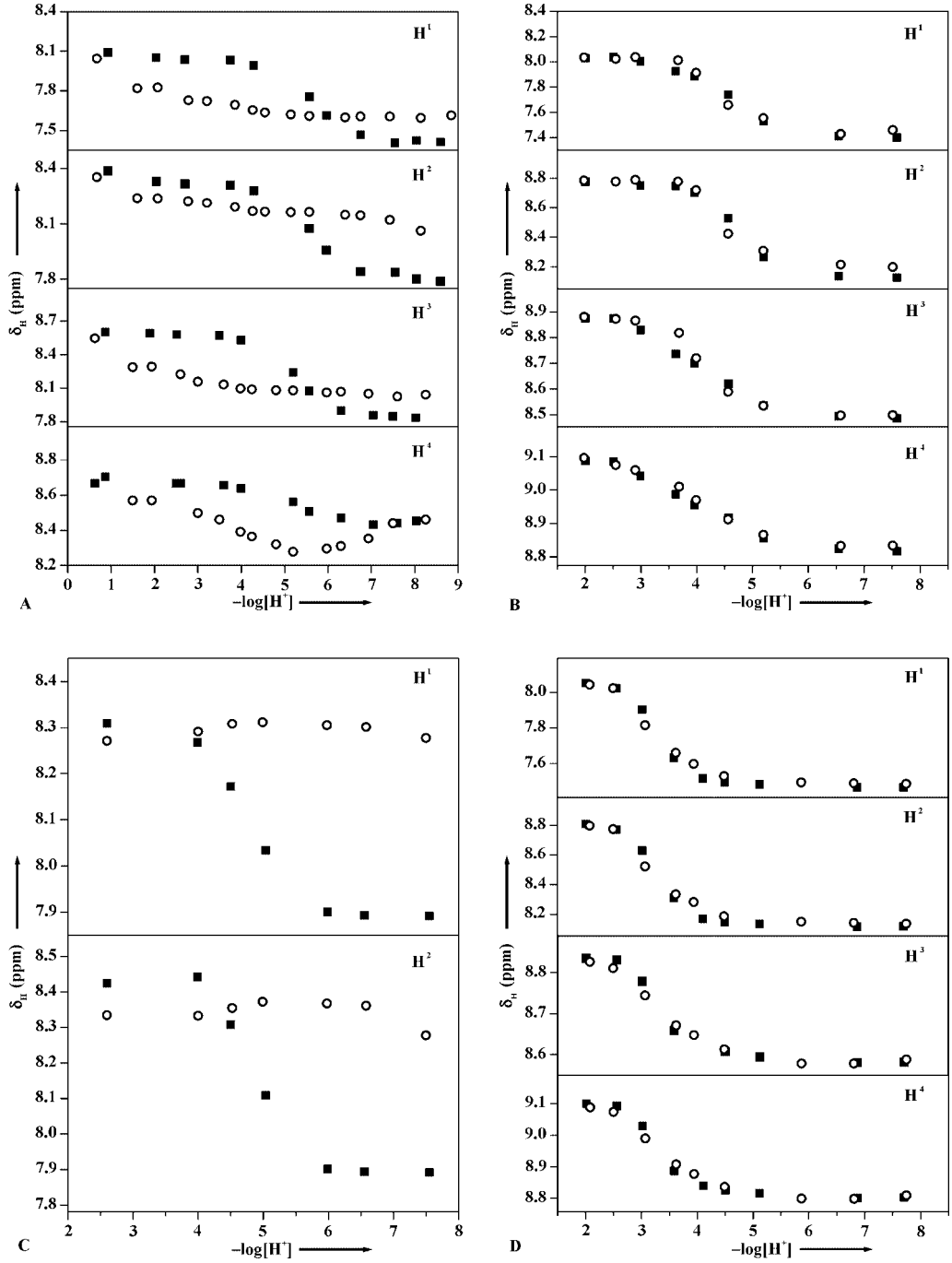


Figure 8. Comparison of ^{1}H NMR chemical shifts of HL^1 (A), HL^2 (B), H_2L^3 (C) and L^4 (D) as a function of –log[H^+] for the free ligands (filled squares) and for the ligands in the ternary mixtures with $[Zn(cyclen)]^{2+}$ (open circles; see Figure 9 for assignment).

at δ = 2.95 ppm can be attributed to free cyclen and the protonated ternary [Zn(Hcyclen)(HL^n)] and [Zn(Hcyclen)(L^n)] complexes in fast chemical exchange. This assignment corresponds with the integration of the ^{1}H NMR signals, as the relative intensities of the singlet at $\delta \approx$ 2.95 ppm {suggested for species protonated at the cyclen amino groups, such as free cyclen and [Zn(Hcyclen)(HL^n)] and [Zn(Hcyclen)(L^n)] complexes} and the signals of the deprotonated ternary complex {[Zn(cyclen)(L^n)], multiplets at $\delta \approx$ 2.8 ppm} nicely correlate with the speciation suggested by potentiometry (Figure 7). The signal at δ = 2.95 ppm also appears in the spectrum after addition of picolinic acid to a solution of [Zn(cyclen)]$^{2+}$ at pH ≈ 6.

The formation of ternary complexes with pyridine ligands bound in the axial position is also evident from a small but distinct change of the position of the amine proton signal at $\delta \approx$ 3.5 ppm. Unlike to the binary Zn^{2+}-cyclen system, where δ_H(NH) starts to change with the formation of the hydroxido complex (at pH > 7), in the ternary systems the amine proton signal (Figure 6) moves from pH ≈ 4 due to formation of the deprotonated ternary species (Figure 7). The chemical shift of the amine protons seems to be sensitive to the replacement of the water molecule in the axial position with a hydroxide anion and/or a pyridine ligand.

The changes in the aromatic region of the ^{1}H NMR spectra of both Zn^{2+}-cyclen-H$_x$L^n systems and free ligands H$_x$L^n as the pH changes are plotted in Figure 8. Assignment of the protons is shown in Figure 9. It is clear from the plots of the free acids that distinct breaks of the chemical shifts correspond to deprotonation of the pyridine nitrogen atom. The estimated pK_A values for this reaction correspond to those determined from potentiometry (see Table 2). Deprotonation of the carboxylic groups causes only small changes of the spectra.

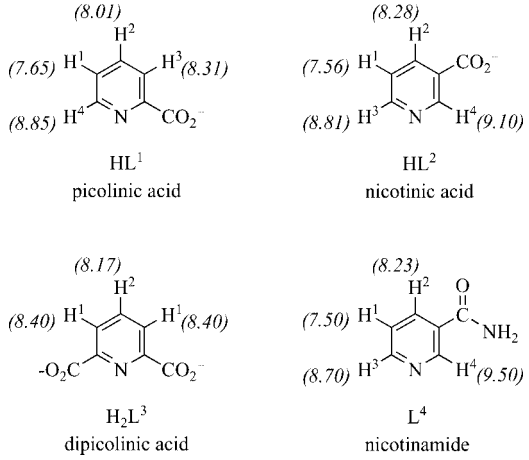


Figure 9. Assignment of the aromatic hydrogen atoms of HL^1, HL^2, H$_2$L^3 and L^4; ^{1}H NMR chemical shifts for the protons (in ppm) in the free ligands are given in parentheses.

The plots of signals of the free ligands and the ligands in the ternary systems with nicotinic acid and nicotinamide are quite similar. This similarity indicates that deprotonation of the nitrogen atom is not significantly influenced by complexation and therefore that the interactions of both the ligands with [Zn(cyclen)]$^{2+}$ and in the acid region with free Zn^{2+} are weak.

A different situation results from an analogous comparison of the systems with picolinic or dipicolinic acids. The chemical-shift changes of of free picolinic acid and its ternary system point to two deprotonation steps and simultaneous coordination: one in the pH range 1–2 and the other at pH = 3–5. The observed shapes of the δ_H vs. pH plots indicate that picolinic acid coordinates in the acid region through the carboxylic group whereas in the pH region 3–5 it forms a chelate through the carboxylate group and the nitrogen atom. This is especially evident from the signal of the H^4 proton, which is strongly influenced by the deprotonation of the pyridine nitrogen atom. In this case, the change in the signal position of the free acid and the ligand in the ternary system in the acid region is significant. For dipicolinic acid, the δ_H vs. pH plot indicates a similarity in the region of pH = 2–4 and, as above, the shape also points to the formation of a chelate. Thus, in both systems Zn^{2+} is hexacoordinate in the [Zn(cyclen)(L^n)] species due to the bidentate coordination of the pyridinecarboxylic acids and tetradentate coordination of cyclen.

Molecular Modelling

The minimal total energies, E_{min}, of both protonated and non-protonated [Zn(Hcyclen)(L^n)] and [Zn(cyclen)(L^n)] species were calculated to estimate an alternative Zn^{2+} coordination by nitrogen or oxygen atoms and also to compare the energies of the protonated and non-protonated ternary complexes (see Table 5). As we concluded from the NMR results, monodentate coordination is found for nicotinic acid and nicotinamide and chelate formation for picolinic and dipicolinic acids. These coordination modes were therefore considered. The coordination sphere was completed with a molecule of water to give a coordination number of five where necessary.

Table 5. Minimised energies calculated for the ternary complexes.

Species	E_{min} [kJ mol^{-1}] Coordination of (L^n)$^{x-}$ through		
	pyridine	oxygen	chelate
[Zn(cyclen)(L^4)]$^{2+}$	30.8	31.0	
[Zn(Hcyclen)(H$_2$O)(L^4)]$^{3+}$	43.9	40.2	
[Zn(cyclen)(L^2)]$^{+}$	29.1	28.8	
[Zn(Hcyclen)(H$_2$O)(L^2)]$^{2+}$	43.5	39.2	
[Zn(cyclen)(L^1)]$^{+}$			46.0
[Zn(Hcyclen)(L^1)]$^{2+}$			43.9
[Zn(cyclen)(L^3)]			58.0
[Zn(Hcyclen)(L^3)]$^{+}$			53.8

The E_{min} values found for [Zn(Hcyclen)(H$_2$O)(L^2)]$^{2+}$ and [Zn(Hcyclen)(H$_2$O)(L^4)]$^{3+}$ are higher than those of [Zn(cyclen)(L^2)]$^{+}$ and [Zn(cyclen)(L^4)]$^{2+}$. As expected, species with fully coordinated cyclen are more stable than those in which one nitrogen atom is protonated (and non-coordi-

nated). Both protonated species prefer to coordinate through the carbonyl oxygen atom rather than through the pyridine nitrogen atom. On the other hand, the E_{min} values of the $[Zn(cyclen)(L^2)]^+$ and $[Zn(cyclen)(L^4)]^{2+}$ are virtually the same for both coordination modes, therefore an equilibrium between interactions through nitrogen and oxygen atoms is assumed in these species. The species $[Zn(Hcyclen)(H_2O)(L^2)]^{2+}$ and $[Zn(Hcyclen)(H_2O)(L^4)]^{3+}$ are present in acid solution (pH ≈ 3–4). There are no significant differences in the chemical shifts of the aromatic proton signals between the free ligands and the ligands in the ternary mixtures in this region, which indicates only a weak interaction between $[Zn(cyclen)]^{2+}$ and HL^2 or L^4.

As mentioned above, the formation of chelates for both $(L^1)^-$ and $(L^3)^{2-}$ is assumed in both protonated ($[Zn(Hcyclen)(L^1)]^{2+}$ and $[Zn(Hcyclen)(L^3)]^+$) and deprotonated ($[Zn(cyclen)(L^1)]^+$ and $[Zn(cyclen)(L^3)]$) species, and these types were included in our treatment. In contrast to the systems with nicotinic acid and nicotinamide, the values of E_{min} in systems containing picolinic and dipicolinic acids are lower for the protonated complexes than for the deprotonated species. This corresponds to NMR titrations in which the presence of the protonated species was confirmed even at pH ≈ 7.

These results support the potentiometric conclusions, which suggest that the formation of an additional chelate in the $[Zn(cyclen)]^{2+}$ moiety destabilizes the tetradentate coordination of cyclen. Thus, the Zn^{2+} complexes with coordination number six seem to be less stable than those with coordination number five.

In addition, we searched for intramolecular hydrogen bonds between the cyclen amino group and the carboxylate groups of the coordinated acids similar to those found in the Zn^{2+}-cyclen-thymine system.[38] The only interaction found was in the $[Zn(cyclen)(L^3)]$ species, with an estimated $H_{amine}\cdots O_{carboxylate}$ distance of around 2.16 Å (which corresponds to a weak hydrogen bond).

Conclusions

The central Zn^{2+} ion in the $[Zn(cyclen)(L^n)]$ complexes of nicotinic acid or nicotinamide is pentacoordinate in the solid state. This corresponds to the known structural motif of analogous complexes containing the $[Zn(cyclen)]^{2+}$ fragment. Picolinic acid forms a $[\{Zn(cyclen)\}_2(\mu\text{-}L^1\text{-}O{:}N,O')]^{3+}$ cation in the solid state, where one zinc atom is pentacoordinate and the other hexacoordinate. A hexacoordinate motif has not yet been observed for the $[Zn(cyclen)]^{2+}$ fragment and is present in only a few complexes with *N*-substituted cyclen derivatives.

The NMR results in solution indicate that Zn^{2+} is also hexacoordinate in the $[Zn(cyclen)(L^n)]$ species with picolinic and dipicolinic acids, and protonated $[Zn(Hcyclen)(HL^n)]$ and $[Zn(Hcyclen)(L^n)]$ species have also been identified. The cyclen ligand in these species should be protonated and only tridentate coordination is assumed from a comparison of pK_A values; the coordination number of the central ion should therefore be five. Unfortunately, all attempts to prepare crystals of the protonated species to confirm this hypothesis failed.

The difference between the stability constants of $[Zn(cyclen)]^{2+}$ and $[Zn(L^{1,3})]$ is 9–10 orders of magnitude and between $[Zn(cyclen)]^{2+}$ and $[Zn(L^2)]^+$ 13 orders of magnitude (Tables 2 and 3). When comparing the stability constants of the binary systems, it is surprising that coordination of H_xL^n to $[Zn(cyclen)]^{2+}$ causes the protonation of cyclen in the $[Zn(Hcyclen)(HL^n)]$ and $[Zn(Hcyclen)(L^n)]$ species even though the $[Zn(Hcyclen)]^+$ ion has not been identified in the binary system. This comparison suggests the preference for a coordination number of five in Zn^{2+} complexes. This corresponds to Hancock's conclusion based on a comparison of the binding properties of $[Zn(cyclen)]^{2+}$ and [Zn(1,5,9-triazacyclododecane)]$^{2+}$ complexes.[39] The structures of the known zinc enzymes[6] also indicate the preference of Zn^{2+} to form only three coordination bonds when anchored in the catalytic site of proteins. From this point of view, the [Zn(1,5,9-triazacyclododecane)]$^{2+}$ complex would be more convenient for modelling the zinc enzymes. On the other hand, the observed protonation of the $[Zn(cyclen)]^{2+}$ fragment could be utilized to model hydrolases in which one basic site is required. In addition, the affinity of $[Zn(cyclen)]^{2+}$ for picolinic and dipicolinic acids indicates potential inhibition properties of their derivatives for zinc enzymes. Generally, the possibility of protonating cyclen in its Zn^{2+} complexes should be taken into account when considering the $[Zn(cyclen)]^{2+}$ fragment as a model for the above enzymes.

Experimental Section

General: The pyridine derivatives were purchased from Fluka and recrystallised from water before use. Cyclen was obtained from Strem and purified by vacuum sublimation for potentiometric measurements. Elemental analyses were performed at the Department of Organic Chemistry, P. J. Šafárik University, Košice. The zinc content was determined by complexometric titration with Na_2H_2edta after sample mineralisation. The water used in physicochemical measurements was purified using the Milli-Q System (Millipore). $Zn(ClO_4)_2\cdot 6H_2O$ and $Zn(NO_3)_2\cdot 6H_2O$ (Lachema) were recrystallised from slightly acidified water. The other chemicals were of analytical purity.

Preparation of the Zinc(II) Complexes

$[Zn(cyclen)(NO_3)]ClO_4$: A solution of cyclen (345 mg, 2.00 mmol) in water (20 mL) was acidified with dilute HNO_3 [378 mg of concentrated acid (6.00 mmol) in 20 mL of water]. An aqueous solution (20 mL) of zinc perchlorate hexahydrate (745 mg, 2.00 mmol) was then added, and the pH was adjusted with 1 M NaOH to 5.9. The mixture was allowed to stand for 24 h and then an equal volume of ethanol was added. Colourless crystals were obtained after a few days. Yield: 0.55 g (69%). $C_8H_{20}ClN_5O_7Zn$ (399.11): calcd. C 24.07, H 5.05, N 17.55, Zn 16.38; found C 20.38, H 4.42, N 13.52, Zn 17.85.

$[\{Zn(cyclen)\}_2(\mu\text{-}L^1\text{-}N{:}O,O')](ClO_4)_3\cdot H_2O$: Zinc perchlorate hexahydrate (172 mg, 0.462 mmol) in water (5 mL) was slowly added to an aqueous solution (5 mL) of cyclen (80 mg, 0.462 mmol). After standing for 24 h, an equimolar amount of picolinic acid (58 mg,

0.462 mmol) in water (5 mL) was added, and, after a further 2 h, the pH was adjusted with 1 M NaOH to 5.6. The solution was left to stand in air for 24 h, then acetone (10 mL) was added, and colourless crystals were obtained after a few days. Yield: 0.14 g (66%). $C_{22}H_{46}Cl_3N_9O_{15}Zn_2$ (913.78): calcd. C 29.50, H 4.95, N 14.07, Zn 14.60; found C 29.14, H 4.78, N 12.95, Zn 13.75.

[{Zn(cyclen)}$_2$(μ-L^2-*N*,*O*)](ClO$_4$)$_3$: Zinc perchlorate hexahydrate (86 mg, 0.232 mmol) in water (3 mL) was slowly added to an aqueous solution (7 mL) of cyclen (40 mg, 0.232 mmol). After standing for 24 h, an equimolar amount of nicotinic acid (29 mg, 0.232 mmol) in water (10 mL) was added, and the solution was left to stand at room temperature (pH = 4.3). Colourless and transparent crystals were obtained after several days. Yield: 0.065 g (63%). $C_{22}H_{44}Cl_3N_9O_{14}Zn_2$ (895.76): calcd. C 29.50, H 4.95, N 14.07, Zn 14.60; found C 29.49, H 4.51, N 12.99, Zn 15.75.

[Zn(cyclen)(L^4-*O*)](ClO$_4$)$_2$: Zinc perchlorate hexahydrate (172 mg, 0.462 mmol) in water (5 mL) was slowly added to an aqueous solution (5 mL) of cyclen (80 mg, 0.462 mmol). After standing for 24 h, an equimolar amount of nicotinamide (56 mg, 0.462 mmol) in water (5 mL) was added. After another 2 h, once the pH of the solution had stabilized at 5.9, acetone (10 mL) was added. The colourless product crystallised over several days. Yield: 0.19 g (74%). $C_{14}H_{26}Cl_2N_6O_9Zn$ (558.68): calcd. C 30.10, H 4.69, N 15.04 Zn 11.70; found C 29.90, H 4.39, N 14.33, Zn 10.93.

X-ray Crystallography: Colourless prismatic crystals of the complexes selected from the bulk before filtration were used for data collection. Unit cell determination and data collection were carried out with a Nonius KappaCCD diffractometer using graphite-monochromated Mo-K_α radiation (λ = 0.71070 Å). The phase problem was solved with SIR-97[40] and structure refinement was carried out by the full-matrix least-squares method on F^2 using the SHELXL-97[41] program. All non-hydrogen atoms were refined anisotropically. Experimental crystallographic parameters of all complexes are listed in Table 6. CCDC-633924 for [Zn(cyclen)(NO$_3$)]ClO$_4$, -633926 for [{Zn(cyclen)}$_2$(μ-L^1-*O:N,O′*)](ClO$_4$)$_3$·H$_2$O, -634730 for [{Zn(cyclen)}$_2$(μ-L^2-*N*,*O*)](ClO$_4$)$_3$ and -633925 for [Zn(cyclen)(L^4-*O*)](ClO$_4$)$_2$ contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

^{1}H NMR Titrations: ^{1}H NMR spectra were recorded with a Varian–Unity Inova 400 spectrometer. Samples were prepared in H_2O and the spectra recorded relative to a D_2O insert containing 0.5% *t*BuOH (δ = 1.25 ppm) as reference and lock. Four FIDs were accumulated for each spectrum using a standard-transmitter water-presaturated pulse sequence. The initial volume of solutions for each titration point was 0.7 mL. In the titration experiments, solutions contained aliquots of Zn(NO$_3$)$_2$·6H$_2$O, cyclen and an appropriate ligand. The initial concentration of individual species was 0.1 M for the ternary Zn^{2+}-cyclen-HL^{1,2} and Zn^{2+}-cyclen-HL^4 mixtures and 0.05 M for the Zn^{2+}-cyclen-H$_2$L^3 system. The required pH values of the solutions were attained by adding aqueous 0.1 M NaOH or 0.1 M HNO$_3$ solutions.

Potentiometric Titrations: Stock solutions of pyridine derivatives were prepared by dissolving solid samples in deionised water. Their concentrations were calculated from the weight of samples and agreed well with those found in calculations of protonation constants of the ligands. The cyclen stock solution (approx. 0.02 M) was prepared by dissolving the solid in deionised water followed by addition of a standardised HNO$_3$ solution (final concentration of acid in the stock solution of cyclen was 0.06 M); the exact concentration of cyclen was calculated together with the determination of protonation constants. The preparation of standard solutions, titrations and calculation were performed as published previously.[42] Potentiometric measurements were carried out with a PHM 84 pH-meter, ABU 80 autoburette and a GK 2401 B combination electrode (Radiometer) in a glass vessel (10 mL) thermostatted at 25.0 ± 0.1 °C at an ionic strength I(KNO$_3$) of 0.1 M. An inert atmosphere was ensured by a constant flow of argon saturated with the solvent vapour. For determination of the protonation constants, the concentration of ligands was 0.004 M and the initial volume was 5 mL. In the case of Zn^{2+} complexes with HL^1, HL^2, H$_2$L^3 and L^4, the volume of the initial solution was also 5 mL and the ligand concentration ranged from 0.002 to 0.004 M. The Zn^{2+}/ligand ratio was 2:1, 1:1, 1:2 and 1:4, with [Zn^{2+}] = 0.001–0.004 M. At least three parallel titrations were carried out for each ratio; each titration consisted of 40–50 points. The total dataset consisted of approximately 500 data points per given Zn-H$_x$L^n system. Equilibration was slow for the Zn^{2+}-cyclen and ternary mixtures; therefore the out-of-cell method was used. Around 4 h was required to attain equilibrium in the binary Zn^{2+}-cyclen system. For the ter-

Table 6. Crystallographic parameters of the studied complexes.

	[Zn(cyclen)(NO$_3$)]ClO$_4$	[{Zn(cyclen)}$_2$(μ-L^1-*O:N,O′*)](ClO$_4$)$_3$·H$_2$O	[{Zn(cyclen)}$_2$(μ-L^2-*N*,*O*)](ClO$_4$)$_3$	[Zn(cyclen)(L^4-*N*)](ClO$_4$)$_2$
Empirical formula	$C_8H_{20}ClN_5O_7Zn$	$C_{22}H_{48}Cl_3N_9O_{15}Zn_2$	$C_{22}H_{44}Cl_3N_9O_{14}Zn_2$	$C_{14}H_{26}Cl_2N_6O_9Zn$
M_r	399.11	913.78	895.75	558.68
Crystal colour	colourless	colourless	colourless	colourless
Crystal dimensions [mm]	unknown	0.13 × 0.15 × 0.23	0.3 × 0.25 × 0.25	0.15 × 0.25 × 0.55
Crystal system	monoclinic	monoclinic	monoclinic	monoclinic
Space group	$P2_1/n$	$P2_1/c$	$P2_1/c$	$P2_1/n$
Lattice parameters	a = 8.8470(3) Å	a = 8.6920(4) Å	a = 15.5830(2) Å	a = 8.8030(2) Å
	b = 14.9780(9) Å	b = 22.9190(8) Å	b = 17.1720(2) Å	b = 18.7180(5) Å
	c = 12.0220(6) Å	c = 18.9450(8) Å	c = 14.4250(2) Å	c = 13.3320(4) Å
	β = 93.512(3)°	β = 90.747(3)°	β = 114.4890(9)°	β = 96.0560(15)°
V [Å]^3	1590.05(14)	3773.75(3)	3512.76(8)	2184.51(10)
Z	4	4	4	4
$D_{calcd.}$ [g cm^{-3}]	1.667	1.612	1.694	1.699
μ [mm^{-1}]	1.753	1.558	1.670	1.43
$2\theta_{max}$ [°]	50.01	50.05	55.00	55.03
Measured reflections	11268	17483	57756	28957
Independent reflections [$I > 2\sigma(I)$]	2814	6565	8048	4157
R_1	0.0471	0.1211	0.039	0.0542
wR_2	0.1213	0.3286	0.1021	0.1417

nary systems, the Zn^{2+}-cyclen complex was formed first (overnight equilibration), then a solution of the second ligand was added along with an appropriate amount of hydroxide solution. The final reaction mixture was left to stand for at least 15 h for equilibration. The molar ratios used were 1:1 for Zn^{2+}-cyclen and 1:1:1 for the ternary systems, with concentrations of the components in the range 0.003–0.004 M. The initial volume of the solution (before addition of the hydroxide solution) for each titration point was 1 mL. The titrations were performed at least in duplicate with 25 points per titration. A typical dataset consisted of around 70 data points for each system. Precise calibration of the electrode was carried out by titration of 0.03 M HNO_3 with 0.2 M KOH in the range $-\log[H^+] = 1.8–12.0$, with the pH-meter yielding E values. The relation between E and $-\log[H^+]$ is expressed by Equation (1) where the term E_0 contains the standard potentials of the electrodes used and the contribution of inert ions to the liquid-junction potential. S corresponds to the Nernstian slope, the value of which should be close to the theoretical value, and $j_1 \times [H^+]$ and $j_2 \times [OH^-] = j_2 \times K_w/[H^+]$ are contributions of the H^+ and OH^- ions, respectively, to the liquid-junction potential. The pK_w value was 13.78 [Equation (1)].

$$E = E_0 - S \times (-\log[H^+]) + j_1 \times [H^+] + j_2 \times K_w/[H^+] \quad (1)$$

It is clear that j_1 and j_2 cause deviation from a linear dependence between E and $-\log[H^+]$ only in strongly acid and strongly alkaline solutions. The protonation and stability constants were calculated with the OPIUM software package.[43]

Molecular Modelling Calculations: The calculations were carried out with a Pentium® IV 2.0 GHz PC using Hyperchem Pro™ version 7.04.[44] The Conformational Search module was used to find the lowest energy conformation of the coordinated cyclen, analyzing the systems in 1000 cycles. The energy of the systems in vacuo was minimised using molecular mechanics employing the MM+ force field and the Polak–Ribiere (conjugate gradient) algorithm, ending at an energy gradient of 0.01 kJ mol^{-1}. The geometry was optimised without any constraints by allowing all atoms, bonds and dihedral angles to change simultaneously.

Supporting Information (see footnote on the first page of this article): Colour versions of Figures 5 and 7; selected geometric parameters found in the studied crystal structures; arrangement of the chelate rings in the $[\{Zn(cyclen)\}_2(\mu\text{-}L^1\text{-}O{:}N,O')]^{3+}$ cation; distribution of the complex species formed in the Zn^{2+}-H_xL^n systems as a function of $-\log[H^+]$; distribution of the complex species formed in the Zn^{2+}-cyclen system as a function of $-\log[H^+]$.

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