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Supplementary material

Kinetics of Zn(II) complexation by a ditopic phenanthroline-azamacrocyclic scorpiand-like receptor

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Fig. S1. Distribution diagram for the protonation of L with the chemical shift of its spectra in D_2O to several pD values

Fig. S2. UV-Vis spectral variations occurring upon addition of 2 eq of Zn^{2+} to a solution of compound L (traced line) at pH = 8. The spectrum obtained immediately after (8s) the addition of the metal (0) is indicated. (Conditions: [L] = 2.5×10^{-5} M; [NaCl] = 0.15 M; 5% universal buffer).

Fig. S3. Distribution diagrams of the species for the L:Zn^{2+} systems as a function of pH in aqueous solution in 0.15 M NaCl at 298.1 K; [L] = 1×10^{-3} M. (a) [Zn^{2+}] = 1×10^{-3} M; (b) [Zn^{2+}] = 2×10^{-3} M.

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Fig. S5. Fluorescence kinetic traces at 365 nm (λ_{exc} = 268 nm) for 1:1 and 2:1 molar ratios (Zn^{2+}:L). (Conditions: [L] = 6×10^{-6} M; [NaCl] = 0.15 M; 5% universal buffer, pH = 8).

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Fig S7. Plot of the crystal structure of a Zn^{2+} complex of a related monoprotonated macrocycle without fluorophore group ($[Zn(HL)Br][ZnBr_4]_2\cdot H_2O$) showing the coordination of the metal ion tto the macrocycle.

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S1. Synthesis of compound L.

5-(2-Aminoethyl)-2,5,8-triaza[12]-2,6-pyridinophane[1] (0.29 g, 1.16 mmol) and 4-carboxaldehyde-2,9-phenanthroline (0.241 g, 1.16 mmol) were dissolved in 40 mL anhydrous ethanol and the mixture was stirred for 2 h at room temperature. $NaBH_4$ (0.438 g, 11.6 mmol) was then added and the resulting solution stirred for 2 h at room temperature. The ethanol was removed under reduced pressure. The resulting residue was treated with H_2O (10 mL) and extracted with CH_2Cl_2 (3x20 mL). The organic phase was removed at reduced pressure, and the resulting residue was dissolved in ethanol and precipitated as hydrochloride salt of L in 60 % yield.
^{1}H NMR (500 MHz, D_2O): δ (ppm) = 8.69 (d, J = 5 Hz, 1H), 8.67 (d, J = 4 Hz, 1H), 8.12 (d, J = 8 Hz, 1H), 7.86 (t, J = 8 Hz, 1H), 7.67 (d, J = 4 Hz, 1H), 7.58 (dd, J_1 = 8 Hz, J_2 = 5 Hz, 1H), 7.52 (d, J = 8 Hz, 1H), 7.35 (d, J = 8 Hz, 2H), 7.33 (d, J = 8 Hz, 1H), 4.62 (s, 2H), 4.55 (s, 4H), 3.44 (t, J = 7 Hz, 2H), 3.26-3.18 (m, 4H), 3.09 (t, J = 7 Hz, 2H), 2.89 (t, J = 5 Hz, 4H).
^{13}C NMR (125 MHz, D_2O): δ (ppm) = 149.35, 148.95, 147.59, 141.41, 140.31, 139.89, 139.65, 128.01, 126.03, 124.28, 122.06, 121.56, 51.01, 50.56, 49.57, 46.82, 45.94, 44.18.
Calc for $C_{26}N_7H_{31}\cdot 4HCl\ 6.2H_2O$: C, 44.6, H, 6.8, N, 14.0. Found: C, 44.6, H, 6.7, N, 14.1. *MS (FAB) m/z* 442 $[M+H]^+$.

S2. NMR measurements.

The ^{1}H and ^{13}C spectra were recorded on a Bruker AV 500 spectrometer at 500 MHz for ^{1}H and 125.72 MHz for ^{13}C. The chemical shifts were recorded in ppm. All spectra were recorded at room temperature and the concentration of $L\cdot 4HCl\ 6.2H_2O$ was 2 mM in D_2O and the $[Zn^{2+}]$ was kept between 2-4 mM. The pD was adjusted with a concentrated solution of DCl or NaOD in D_2O.

S3. EMF measurements

The potentiometric titrations were carried out at 298.1 ± 0.1 K using NaCl 0.15 M as supporting electrolyte. The experimental procedure (burette, potentiometer, cell, stirrer, microcomputer, etc.) has been fully described elsewhere.[2] The acquisition of the EMF data was performed with the computer program PASAT.[3] The reference electrode was an Ag/AgCl electrode in saturated KCl solution. The glass electrode was calibrated as a hydrogen ion concentration probe by titration of previously standardized amounts of HCl with CO^{2-}-free NaOH solutions and the equivalent point determined by the Gran'smethod,[4] which gives the standard potential, $E°$', and the ionic product of water (pKw = 13.73(1)).
The computer program HYPERQUAD was used to calculate the protonation and stability constants.[5] The HYSS[6] program was used to obtain the distribution diagrams. The pH range investigated was 2.5-11.0. In the binary Zn^{2+}-L system, the concentration of Zn^{2+} and the ligand ranged from $1x10^{-3}$ to $5x10^{-3}$ mol dm^{-3} with Zn^{2+}:L molar ratios varying from 2:1 to 1:2. The titration curves for each system (at least two titrations, ca. 200 experimental) were treated either as a single set or as separated curves without significant variations in the values of the stability

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4 (a) G. Gran, Analyst, 1952, **77**, 661; (b) F. J. Rossotti, H. Rossotti, J. Chem. Educ., 1965, 42, 375.
5 P. Gans, A. Sabatini, A. Vacca, *Talanta,* 1996, **43**, 1739.
6 P. Gans, *Programs to determinethe distribution of Species in multiequilibria systems from the stability constants and mass balance equations*.

constants. When more than one model could fit the experimental data, the most reliable chemical model was chosen by performing F tests at the 0.05 confidence level.[7]

S4. UV-Vis and fluorescence measurements

Solutions were prepared using Millipore water, with NaCl 0.15 M as supporting electrolyte, and using 5% (v/v) of the universal buffer of Theorell and Stenhagen [8] at the appropriate pH value. The absorption and emission measurements were carried out at 294.1 K using both a CARY 100Bio spectrophotometer and a Jobin Yvon Spex, Fluorolog FL3-22 spectrofluorimeter respectively. In the kinetic experiments, a solution of compound L at pH = 8 was treated with 1 or 2 eq of Zn^{2+}, proceeding from a concentrated solution of the metal at the same pH value. The fluorescence kinetic experiments were recorded by exciting at 268 nm and collecting the emission at 365 nm. The measured spectral changes were analyzed and fitted using the tool SOLVER provided by Microsoft Office EXCEL 2003.

S5. Stopped-flow experiments

The measurements were carried out at 294.1 K with an Applied Photophysics SX20 stopped-flow spectrometer provided with a PDA.1/UV photodiode array. Solutions were prepared with Millipore water, the ionic strength was fixed using NaCl 0.15 M and the pH was controlled by employing 5% (v/v) of the universal buffer of Theorell and Stenhagen at the appropriate pH value. In the experiments, a solution of the compound L at pH = 8 was mixed in the stopped-flow instrument with a solution of Zn^{2+} in 1:1 or 2:1 molar ratio (M:L) at the same pH value. The measured spectral changes were analyzed and fitted to a second order reaction with the tool SOLVER provided by Microsoft Office EXCEL 2003.

Table S1 Logarithms of the stepwise protonation constants for the protonation of L determined in 0.15 mol dm^{-3} NaCl at 298.1 K.

Reaction[a]	L
$H + L = HL$	9.39(2)[b]
$H + HL = H_2L$	7.39(3)
$H + H_2L = H_3L$	5.27(2)
$H + H_3L = H_4L$	3.44(4)
Log β[c]	25.49

[a]Charges omitted for clarity. [b]Values in parentheses are standard deviation in the last significant figure. [c]log $\beta = \Sigma$ log K

Table S2. Logarithms of the cumulative stability constants for the formation complexes of Zn^{2+} with L calculated in 0.15 mol·dm^{-3} NaCl at 298.1 ± 0.1 K.

Reaction [a]	**L**
$H + Zn + L \leftrightarrows ZnHL$	**17.88(4)**
$Zn + L \leftrightarrows ZnL$	**12.48(3)**
$Zn + L + H_2O \leftrightarrows ZnL(OH) + H$	**3.52(6)**
$Zn + L + 2H_2O \leftrightarrows ZnL(OH)_2 + 2H$	**-6.95(5)**
$2Zn + L \leftrightarrows Zn_2L$	**17.60(4)**
$2Zn + L + H_2O \leftrightarrows Zn_2L(OH) + H$	**1.45(5)**
$2H + Zn + 2L \leftrightarrows Zn_2H_2L_2$	**34.27(6)**

[a]Charges omitted. [b]Values in parenthesis show standard deviation in the last significant figure.

7 (a) W. C. Hamilton, *Statistics in Physical chemistry*, The Ronald Press Co., New York, 1964. (b) L. Bologni, A. Sabatini, A. Vacca, *Inorg. Chim. Acta*, 1983, **69**, 71.

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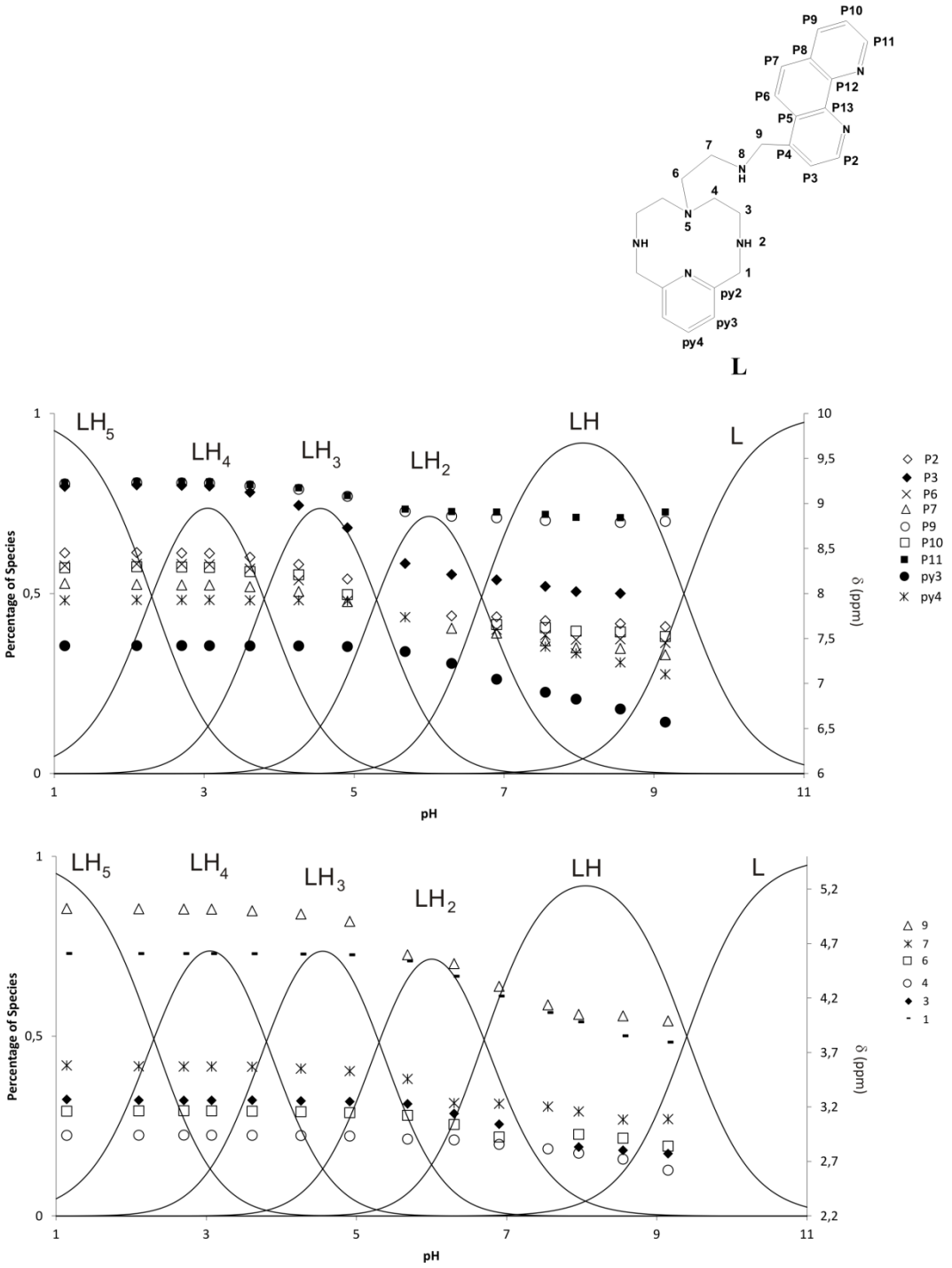
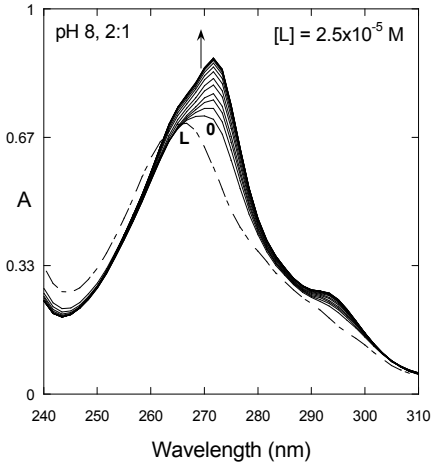


Fig. S1. Distribution diagram for the protonation of L with the chemical shift of its spectra in D_2O to several pD values.



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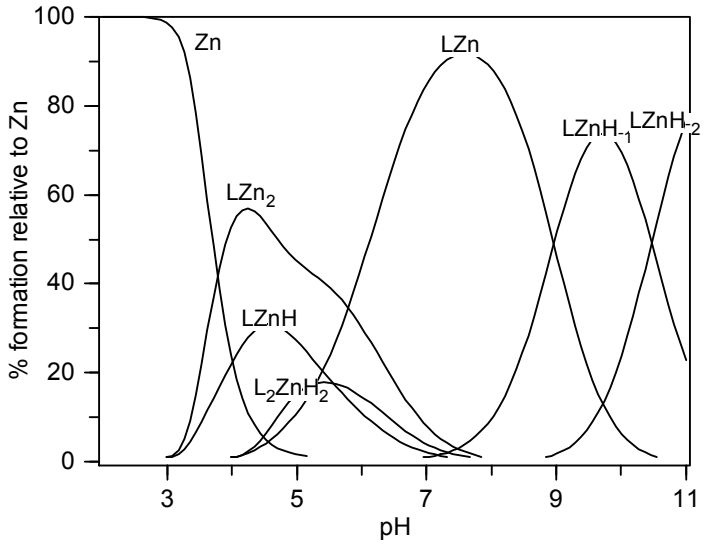
a)



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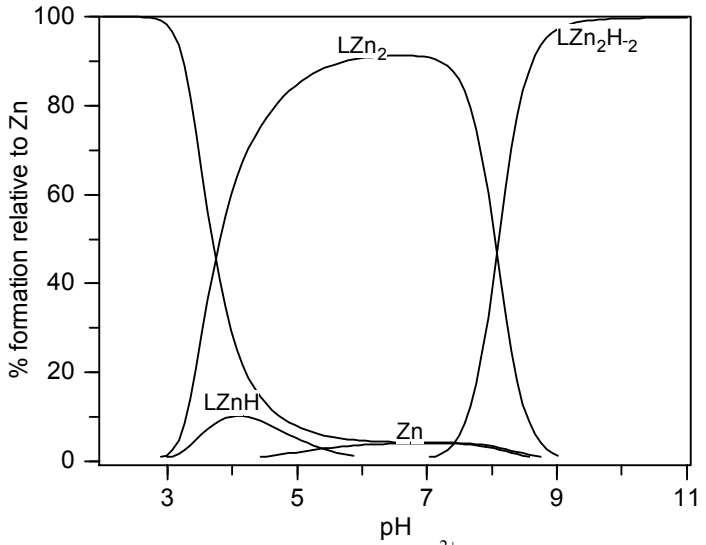

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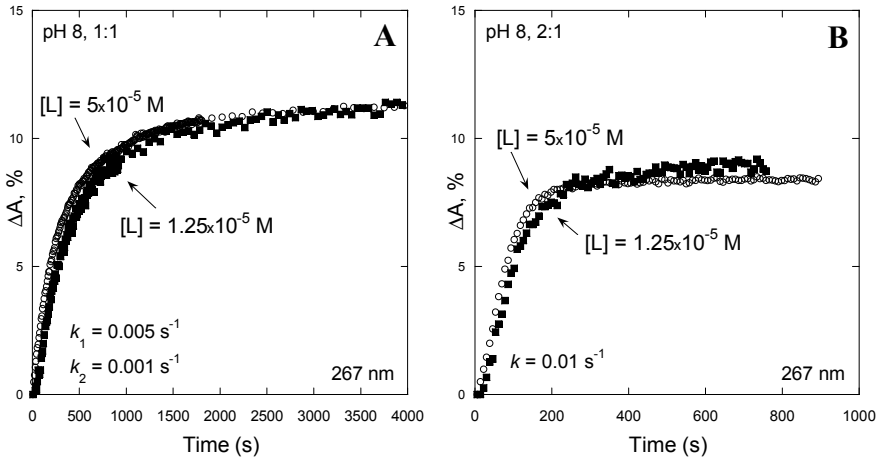

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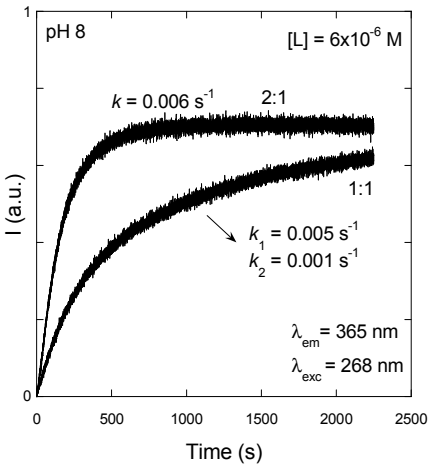

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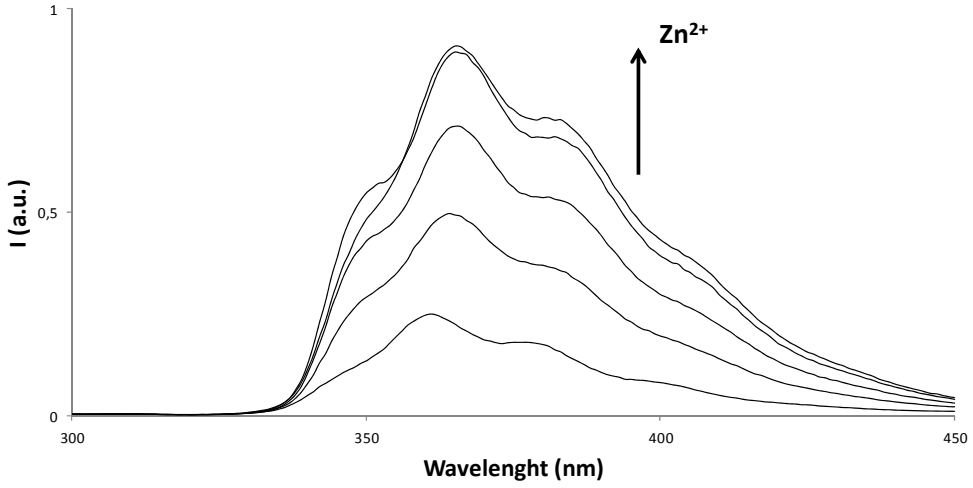

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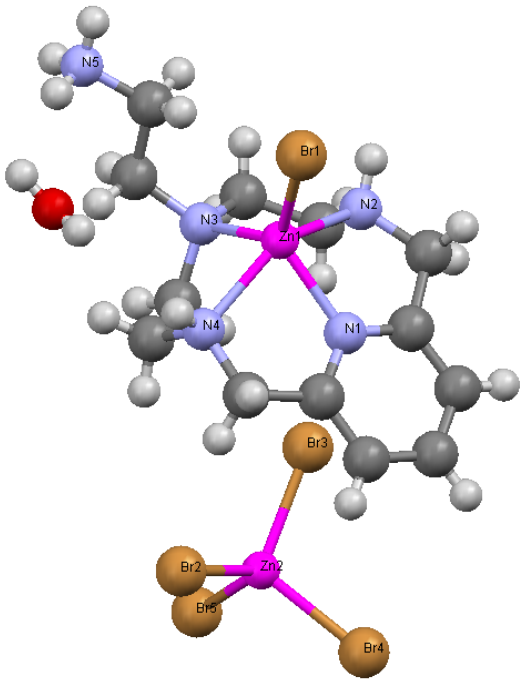

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