J|A|C|S

A R T I C L E S

Coordination and Fluorescence of the Intracellular Zn^{2+} Probe [2-methyl-8-(4-Toluenesulfonamido)-6-quinolyloxy]acetic Acid (Zinquin A) in Ternary Zn^{2+} Complexes

Kym M. Hendrickson, Jason P. Geue, Oska Wyness, Stephen F. Lincoln,* and A. David Ward

Contribution from the Department of Chemistry, University of Adelaide, Adelaide, South Australia 5005, Australia

Received May 13, 2002 ; E-mail: stephen.lincoln@adelaide.edu.au

Abstract: A potentiometric study of the coordination of the fluorophore, 2-methyl-8-(4-toluenesulfonamido)-6-quinolyloxyacetic acid, ^{1}LH$_2$ (the intracellular Zn^{2+} probe, Zinquin A) in its deprotonated form, ^{1}L^{2-}, in Zn^{2+} ternary complexes, [Zn^{n}L^{1}L]^n (where n is the charge of ^{n}L) at 298.2 K in 50% aqueous ethanol (v/v) and I = 0.10 (NaClO$_4$), shows that the formation of [Zn^{n}L^{1}L]^n from [Zn^{n}L]$^{(2+n)+}$ is characterized by log(K_5/dm^3 mol^{-1}) = 8.23 ± 0.05, 4.36 ± 0.18, 8.45 ± 0.10, 10.00 ± 0.06, 11.53 ± 0.06 and 5.92 ± 0.15, respectively, where ^{n}L = ^{2}L − ^{6}L and ^{7}L^{3-} are 1,4,7,10-tetraazacyclododecane, 1,4,8,11-tetraazacyclotetradecane, 1,4,7-triazacyclononane, 1,5,9-triazacyclododecane, tris(2-aminoethyl)amine and nitrilotriacetate, respectively, and K_5 is the stepwise complexation constant. Dissociation of a hydroxo proton from triethanolamine, ^{8}L, occurs in the formation of [Zn^{8}LH$_{-1}$]$^+$ that subsequently forms [Zn^{8}LH$_{-1}$^{1}L]$^-$ for which log(K_5/dm^3 mol^{-1}) = 9.87 ± 0.08. The variation of K_5 and the 5-fold variation of quantum yield of ^{1}L^{2-} as its coordination environment changes in Zn^{2+} ternary complexes are discussed with reference to the use of ^{1}L^{2-} in the detection of intracellular Zn^{2+}.

Introduction

Zinc(II) is widespread in biology and there is much interest in its detection in vivo where its concentration ranges from 10^{-9} mol dm^{-3} in the cytoplasm to 10^{-2} mol dm^{-3} in some vesicles.[1] Accordingly, we developed the Zn^{2+} specific fluorophore 2-methyl-8-(4-toluenesulfonamido)-6-quinolyloxy]acetic acid, Zinquin A, ^{1}LH$_2$ and its ethyl ester, Zinquin E, both of which fluoresce strongly when bound by Zn^{2+},[2,3] and have been widely used in intracellular studies.[4] (An interesting range of other fluorophores designed for Zn^{2+} detection has also been reported.[5]) In vitro studies show that Zinquin A loses protons from its carboxylic acid and sulfonamide groups to form ^{1}L^{2-} which complexes Zn^{2+} in [Zn^{1}L] and [Zn^{1}L$_2$]$^{2-}$, both of which are fluorescent and whose formation we have characterized.[3] {A later potentiometric study in 80:20 DMSO:H$_2$O (w/w) only detected the overall formation of [Zn^{1}L$_2$]$^{2-}$ probably because [Zn^{2+}]$_{total}$/[^{1}L^{2-}]$_{total}$ = 0.5.[6]} However, most intracellular Zn^{2+} is bound by proteins and other bioligands and very little exists in the fully hydrated state whose concentration is reported to be < 10^{-15} mol dm^{-3} in *E. coli*.[7] As all reported studies of intracellular Zn^{2+} using Zinquin A and E show fluorescence consistent with their coordination by Zn^{2+}, it is probable that fluorescent ternary Zn^{2+} complexes are formed where ^{1}L^{2-} is one of the ligands.

Accordingly, it is of interest to establish the extent to which ^{1}L^{2-} coordinates Zn^{2+} in ternary complexes and its fluorescence

(1) Williams, R. J. P. *Polyhedron*, **1987**, *6*, 61. Cunane, S. C. *Zinc: Clinical and Biochemical Significance*; CRC Press: Boca Raton, USA, 1988. Vallee, B. L.; Auld, D. S. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 220. Fausto de Silva; J. J.; Williams, R. J. P. *Biological Chemistry of the Elements*; Oxford University Press: New York, 1991. Vallee, B. L.; Auld, D. S. *Acc. Chem. Res.* **1993**, *26*, 543. Kaim, K.; Schwederski, B. *Bioinorganic Chemistry: Inorganic Elements in the Chemistry of Life*; Wiley: Chichester, 1994.

(2) Mahadevan, I. B.; Kimber, M. C.; Lincoln, S. F.; Gulbis, J. M.; Tiekink, E. R. T.; Ward, A. D.; Betts, W. H.; Forbes, I. J.; Betts, W. H. *Aust. J. Chem.* **1996**, *49*, 561. (^{1}LH$_2$ and its ethyl ester are commercially available from the authors as Zinquin A and Zinquin E, respectively.)

(3) Hendrickson, K.; Rodopoulos, T.; Pittet, P.-A.; Mahadevan, I.; Lincoln, S. F.; Ward, A. D.; Kurucsev, T.; Duckworth, P. A.; Forbes, I. J.; Zalewski, P. D.; Betts, W. H. *J. Chem. Soc., Dalton Trans*. **1997**, 3879.

(4) Coyle, P.; Zalewski, P. D.; Philcox, J. C.; Forbes, I. J.; Ward, A. D.; Lincoln, S. F.; Mahadevan. I.; Roe, A. M. *Biochem. J*. **1994**, *303*, 781. Zalewski, P. D.; Millard, S. H.; Forbes, I. J.; Kapaniris, O.; Slavotinek, A.; Betts, W. H.; Ward, A. D.; Lincoln, S. F.; Mahadevan., I. *J. Histochem. Cytochem.* **1994**, *42*, 877. Zalewski, P. D.; Forbes, I. J.; Seamark, R. F.; Borlinghaus, R.; Betts, W. H.; Lincoln, S. F.; Ward, A. D. *Chem. Biol.* **1994**, *1*, 153. Zalewski, P. D.; Jian, X.; Soon, L. L. L.; Breed, W. G.; Seamark, R. F.; Lincoln, S. F.; Ward, A. D.; Sun, F.-Z. *Reprod. Fertil. Dev.* **1996**, *8*, 1097. Brand, I. A.; Kleineke, J. *J. Biol. Chem*. **1996**, *271*, 1941. Berendji, D.; Kolb-Bachofen, V.; Meyer, K. L.; Grapenthin, O.; Weber, H.; Wahn, V.; Kröncke, K.-D. *FEBS Lett*. **1997**, *405*, 37. Kleineke, J. W.; Brand, I. A. *J. Pharm. Tox. Methods* **1997**, *38*, 181. Haase, H.; Beyersmann, D. *Biometals* **1999**, *12*, 247. Ho, L. H.; Ratnaike, R. N.; Zalewski, P. D. *Biochem. Biophys. Res. Comm*. **2000**, *268*, 148. Qian, W. J.; Aspinwall, C. A.; Battiste, M. A.; Kennedy, R. T. *Anal. Chem*. **2000**, *72*, 711. Pearce, L. L.; Wasserloos, K.; St. Croix, C. M.; Gandley, R.; Levitan, E. S.; Pitt, B. R. *J. Nutrit*. **2000**, *130*, 1467S.

(5) Czarnik, A. W. *Acc Chem. Res*. **1994**, *27*, 302. Walkup, G. K.; Imperiali, B. J. *J. Am. Chem. Soc*. **1997**, *119*, 3443. Walkup, G. K.; Imperiali, B. J. *J. Org. Chem*. **1998**, *63*, 6727. Kimura, E.; Koike, T. *Chem. Soc. Rev.* **1998**, *27*, 179. Hirano, T.; Kikuchi, K.; Urano, Y.; Higuchi, T.; Nagano, T. *Angew Chem. Int. Edn*. **2000**, *39*, 1052. Walkup, G. K.; Burdette, S. C.; Lippard, S. J.; Tsien, R. Y. *J. Am. Chem. Soc.* **2000**, *122*, 5644. Kimber, M. C.; Mahadevan, I. B.; Lincoln, S. F.; Ward, A. D.; Betts, W. H. *Aust. J. Chem*. **2001**, *54*, 43. Pearce, D. A.; Jotterand, N.; Carrico, I. S.; Imperiali, B. *J. Am. Chem. Soc*. **2001**, *123*, 5160. Burdette, S. C.; Walkup, G. K.; Spingler, B.; Tsien, R. Y.; Lippard, S. J. *J. Am. Chem. Soc*. **2001**, *123*, 7831.

(6) Fahrni, C. J.; O'Halloran, T. V. *J. Am. Chem. Soc*. **1999**, *121*, 11 448.

(7) Outten, C. E.; O'Halloran, T. V. *Science* **2001**, *292*, 2488. Hitomi, Y.; Outten, C. E.; O'Halloran, T. V. *J. Am. Chem. Soc*. **2001**, *123*, 8614.
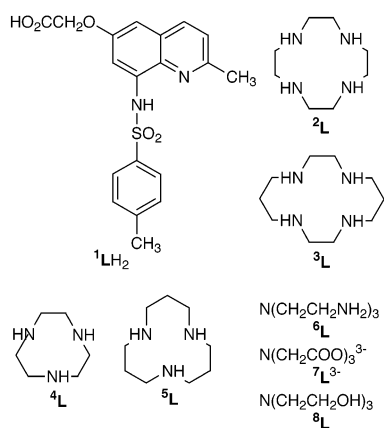
Scheme 1

eq	equilibrium	n	$\log(K_1/dm^3\ mol^{-1})$
(1a)	$Zn^{2+} + {}^{n}L^{2-} = [Zn^{n}L]$	1	9.65^a
(1b)	$Zn^{2+} + {}^{n}L = [Zn^{n}L]^{2+}$	2	15.34 ± 0.02
			$(7.75 \pm 0.05)^b$
		3	14.43 ± 0.03
			$(10.65 \pm 0.13)^b$
		4	12.38 ± 0.07
		5	8.93 ± 0.03
			$(7.60 \pm 0.04)^b$
		6	15.74 ± 0.03
			$(11.13 \pm 0.07)^b$
(1c)	$Zn^{2+} + {}^{n}L^{3-} = [Zn^{n}L]^{-}$	7	10.22 ± 0.03
			$(10.41 \pm 0.04)^b$
			$\log(K_2/dm^3\ mol^{-1})$
(2)	$Zn^{2+} + {}^{n}LH^{+} = [Zn^{n}LH]^{3+}$	2	8.42 ± 0.07
			$(3.60 \pm 0.08)^c$
		4	5.80 ± 0.08
			$(3.74 \pm 0.0)^c$
			$\log(K_3)$
(3)	$Zn^{2+} + {}^{n}L = [Zn^{n}LH_{-1}]^{+} + H^{+}$	8	4.37 ± 0.07
			$(7.45 \pm 0.03)^d$
			$\log(K_4/dm^3\ mol^{-1})$
(4a)	$[Zn^{n}L] + {}^{n}L^{2-} = [Zn({}^{n}L)_2]^{2-}$	1	9.46^a
(4b)	$[Zn^{n}L]^{2+} + {}^{n}L = [Zn({}^{n}L)_2]^{2+}$	4	9.18 ± 0.14
(4c)	$[Zn^{n}L]^{-} + {}^{n}L^{3-} = [Zn({}^{n}L)_2]^{4-}$	7	3.03 ± 0.03
			$\log(K_5/dm^3\ mol^{-1})$
(5a)	$[Zn^{n}L]^{2+} + {}^{1}L^{2-} = [Zn^{n}L^{1}L]$	2	8.23 ± 0.05
		3	4.36 ± 0.18
		4	8.45 ± 0.10
		5	10.00 ± 0.06
			$(6.30 \pm 0.04)^e$
			$(5.67 \pm 0.08)^e$
		6	11.53 ± 0.06
			$(4.54 \pm 0.05)^f$
(5b)	$[Zn^{n}L]^{-} + {}^{1}L^{2-} = [Zn^{n}L^{1}L]^{3-}$	7	5.92 ± 0.15
			$(7.66 \pm 0.15)^e$
			$(3.94 \pm 0.02)^e$
(5c)	$[Zn^{n}LH_{-1}]^{+} + {}^{1}L^{2-} = [Zn^{n}LH_{-1}{}^{1}L]^{-}$	8	9.87 ± 0.08
			$(8.92 \pm 0.08)^g$
(5d)	$[Zn^{n}LH_{-2}] + {}^{1}L^{2-} = [Zn^{n}LH_{-2}{}^{1}L]^{2-}$	8	8.41 ± 0.09

^{a} Ref 3. ^{b} pK_a attributed to proton dissociation of coordinated water. ^{c} pK_as attributed to proton dissociation of ${}^{n}LH^{+}$ in $[Zn^{n}LH]^{3+}$. ^{d} pK_a attributed to dissociation of a hydroxy proton from $[Zn^{n}LH_{-1}]^{+}$. ^{e} pK_as attributed to dissociation of two protons from $[Zn^{5}LH^{1}LH]^{2+}$ and $[Zn^{7}LH^{1}LH]^{-}$. In neither case is the ligand protonation site known with certainty. ^{f} pK_a attributed to dissociation of a proton from $[Zn^{6}L^{1}LH]^{+}$ where the ligand protonation site is not known with certainty. ^{g} pK_a attributed to dissociation of a hydroxy proton from $[Zn^{8}LH_{-1}{}^{1}L]^{-}$ to give $[Zn^{8}LH_{-2}{}^{1}L]^{2-}$.

Table 1. Ligand pK_as Determined in 50% Aqueous Ethanol (v/v) at 298.2 K and $I = 0.10$ Mol dm^{-3} (NaClO$_4$)

ligands	pK_{a1}	pK_{a2}	pK_{a3}
${}^{1}LH_3{}^{+\ a}$	10.01	3.72	1.87
${}^{2}LH_3{}^{3+}$	10.52 ± 0.01	9.10 ± 0.01	1.74 ± 0.18
${}^{3}LH_3{}^{3+}$	11.15 ± 0.02	9.79 ± 0.01	1.56 ± 0.23
${}^{4}LH_3{}^{3+}$	10.32 ± 0.04	6.40 ± 0.02	3.30 ± 0.04
${}^{5}LH_3{}^{3+}$	11.69 ± 0.05	6.80 ± 0.03	2.53 ± 0.03
${}^{6}LH_3{}^{3+}$	10.46 ± 0.03	9.44 ± 0.02	8.16 ± 0.03
${}^{7}LH_3$	9.02 ± 0.04	3.11 ± 0.05	2.76 ± 0.06
${}^{8}LH^{+}$	7.43 ± 0.01^b		

^{a} Ref 3. pK_{a1}, pK_{a2}, and pK_{a3} are assigned to the sulfonamide, carboxylic and quinolinium protons, respectively. ^{b} pK_a of the protonated amine function.

is affected by the presence of other ligands. Thus, we have studied the formation and fluorescence of ternary Zn^{2+} complexes of ${}^{1}L^{2-}$ and the well-characterized tridentate ${}^{4}L$ and ${}^{5}L$ and tetradentate ${}^{2,3,6}L$, ${}^{7}L^{3-}$, and ${}^{8}L$ ligands (Scheme 1) that bind Zn^{2+} strongly. These ligands permit the bidentate coordination of ${}^{1}L^{2-}$ that requires either 5- or 6-coordination of Zn^{2+}, which is encompassed by its normal coordination number range. Although these ligands do not usually occur in biology, their mix of nitrogen and oxygen donor atoms, their macrocyclic and tripodal structures and, in the case of ${}^{7}L^{3-}$, a difference in charge generates a range of environments in which to study the coordination of ${}^{1}L^{2-}$ and its fluorescence. These environmental variations are probably less than those experienced by protein bound intracellular Zn^{2+} which are expected to reflect a range of binding sites and changes in the frequency of their occurrence as the proteome varies with cell type. However, the simpler systems studied here allow quantitative characterization of all species existing in solution including their fluorescence, and provide a guide to the ability of ${}^{1}L^{2-}$ to compete for coordination sites on Zn^{2+} and the concomitant changes in its fluorescence.

Results and Discussion

Formation of Binary and Protonated Binary and Bis Complexes. The potentiometrically determined pK_as for the triprotonated ligands are collected in Table 1. The pK_as for ${}^{2-6}LH_3{}^{3+}$, ${}^{7}LH_3$, and ${}^{8}LH^{+}$ show the trends anticipated from earlier studies,[8] but it was necessary to determine them in 50% aqueous ethanol (v/v) for this study. (Although ${}^{1}LH_2$ and ${}^{1}LH^{-}$ are sufficiently water soluble to give the low concentrations required for biological use, they are only sufficiently soluble

in 50% aqueous ethanol (v/v) at the concentrations required for potentiometric determination of ternary complex stability.) The formation of binary, protonated binary, bis and ternary Zn^{2+} complexes is shown in eqs 1–5, where $K_1 - K_5$ are stepwise complexation constants (Table 2).

Although Zn^{2+} complexes formed by ${}^{2}L - {}^{6}L$ and ${}^{7}L^{3-}$ in water have also been previously reported,[9,10] they have not been characterized under the conditions of this study. The equilibria and potentiometrically determined complexation constants for the binary complexes are shown in Table 2 (eqs 1a − 3) together with pK_as for coordinated water (except for $[Zn^{4}L]^{2+}$ where appearance of a precipitate at pH 8.5, and thought to be

(8) Smith, R. M.; Martell, A. E. *Critical Stability Constants*; Plenum Press: New York, 1975; Vol. 2.

(9) Zompa, L. J.; *Inorg. Chem.* **1978**, *17*, 2531. Desreux, J. F.; Merciny, E.; Loucin, N. C.; *Inorg. Chem.* **1981**, *20*, 987. Micheloni, M.; Sabatini, A.; Paoletti, P. *J. Chem. Soc., Perkin Trans. 2* **1978**, 828. Yang, R.; Zompa, L. J.; *Inorg. Chem.* **1976**, *15*, 1500. Christensen, J. J.; Izatt, R. M.; Wrathall, D. P.; Hansen, L. D. *J. Chem. Soc. (A)* **1969**, 1212.

(10) Kodama, M.; Kimura, E. *J. Chem. Soc., Dalton Trans.* **1977**, 2269. Kimura, E.; Shiota, T.; Kioke, T.; Shiro, M.; Kodama, M. *J. Am. Chem. Soc.* **1990**, *112*, 580.

[Zn^{4}L(OH)]$^+$, precluded a pK_a determination). Generally, as the denticity of the ligand increases so does the stability of the binary complex formed. Superimposed on this are ligand stereochemical restraints as indicated by [Zn^{2}L]$^{2+}$ being more stable than [Zn^{3}L]$^{2+}$, and [Zn^{4}L]$^{2+}$ being more stable than [Zn^{5}L]$^{2+}$. In [Zn^{2}L]$^{2+}$, ^{2}L folds so that the remaining coordination sites are cis to each other while they are trans to each other in [Zn^{3}L]$^{2+}$, assuming six-coordination.[11] This stereochemical difference probably accounts for the large difference in the pK_as of the coordinated water in the two complexes, and also for the 4 orders of magnitude greater stability of the ternary complex, [Zn^{2}L^{1}L]$^{2-}$ (Table 2) compared with that of [Zn^{3}L^{1}L]$^{2-}$ arising from the greater resistance of ^{3}L to folding to allow ^{1}L^{2-} to become bidentate in the latter complex as discussed below. The stereochemistries of [Zn^{6}L]$^{2+}$, [Zn^{7}L]$^-$ and [Zn^{8}LH$_{-1}$]$^+$ are probably similar because of the tripodal tetradentate nature of ^{6}L, ^{7}L^{3-}, and ^{8}LH$_{-1}$$^-$. It is probable that the higher stability of [Zn^{6}L]$^{2+}$ arises in part because the borderline hard acid Zn^{2+} coordinates the four softer base amine nitrogens of ^{6}L more strongly than the three harder base donor oxygens of ^{7}L^{3-} and ^{8}LH$_{-1}$$^-$.

Two protonated complexes are formed, [Zn^{2}LH]$^{3+}$ and [Zn^{4}LH]$^{3+}$, where an amine nitrogen is protonated in each case with a consequent decrease in ligand denticity to three and two, respectively, and a corresponding decrease in stabilities by comparison with those of their unprotonated precursors. The proton lost from [Zn^{8}L]$^{2+}$ in the formation of [Zn^{8}LH$_{-1}$]$^+$ (eq 3) may either come from coordinated water or a hydroxy group of triethanolamine. The bis complexes, [Zn(^{1}L)$_2$]$^{2-}$, [Zn(^{4}L)$_2$]$^{2+}$, and [Zn(^{7}L)$_2$]$^{4-}$ are also formed as shown in eq 4a−c. (The bidentate 6-methoxy-(8-*p*-toluenesulfonamido)quinoline bidentate ligand that closely ressembles ^{1}L^{2-} forms a tetrahedral Zn^{2+} bis complex in the solid state.[12]) Although all donor groups of the ligands are assumed to be coordinated in the first two bis complexes, this cannot be the case in the third if Zn^{2+} retains a coordination number of six. It appears that one or both ligands in [Zn(^{7}L)$_2$]$^{4-}$ exercise less than their maximum denticities of four.

Formation of Ternary Complexes. Ligands ^{2}L − ^{6}L, ^{7}L^{3-}, and ^{8}L participate in the formation of the ternary complexes [Zn^2$^-$^{6}L^{1}L], [Zn^{7}L^{1}L]$^{3-}$, [Zn^{8}LH$_{-1}$^{1}L]$^-$, and [Zn^{8}LH$_{-2}$^{1}L]$^{2-}$ as shown in eq 5a−d in Table 2. For [Zn^{6}L^{1}L], K_5 for the complexation of ^{1}L^{2-} is larger than K_1 for [Zn^{1}L], K_5 for [Zn^{5}L^{1}L] and [Zn^{8}LH$_{-1}$^{1}L]$^-$ are similar to K_1 for [Zn^{1}L], and [Zn^2$^-$^{4}L^{1}L] and [Zn^{7}L^{1}L]$^{3-}$ are smaller. There are two sites available for substitution by ^{1}L^{2-} in the first coordination spheres of [Zn^{2,3,6}L], [Zn^{7}L]$^-$, [Zn^{8}LH$_{-1}$]$^+$, and [Zn^{8}LH$_{-2}$], and three in those of [Zn^{4,5}L] assuming Zn^{2+} to be six-coordinate, so that statistically the probability of substitution by ^{1}L^{2-} should be less favored than on [Zn(H$_2$O)$_6$]$^{2+}$. To varying extents, it is probable that the effects of steric hindrance and changes in electron density at Zn^{2+} superimpose on the statistical effect. The sequence of increasing stabilities [Zn^{7}L^{1}L]$^{3-}$ < [Zn^{8}LH$_{-2}$^{1}L]$^{2-}$ < [Zn^{8}LH$_{-1}$^{1}L]$^-$ is consistent with a similar sequence of increasing electrostatic attraction between the binary complex precursors of these stereochemically similar ternary complexes and ^{1}L^{2-}.

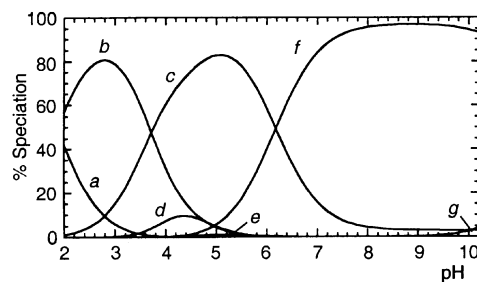(11) Kato, M.; Ito, T. *Inorg. Chem.* **1985**, *24*, 509. Thöm, V. J.; Hosken, G. D.; Hancock, R. D. *Inorg. Chem.* **1985**, *24*, 3378.
(12) Nasir, M. S.; Fahrni, C. J.; Suhy, D. A.; Kolodsick, K. J.; Singer, C. P.; O'Halloran, T. V. *J. Biol. Chem.* **1999**, *4*, 775.

Figure 1. Speciation plot for a 50% aqueous ethanol (v/v) solution 1.03 × 10^{-4} mol dm^{-3} in [^{2}L]$_{total}$, 4.58 × 10^{-5} mol dm^{-3} in [Zn^{2+}]$_{total}$ and 9.84 × 10^{-6} in [^{1}LH$_n^{(n-2)+}$]$_{total}$ at 298.2 K and I = 0.10 (NaClO$_4$). Species are shown as percentages where 100% = [^{1}LH$_n^{(n-2)+}$]$_{total}$. (a) = ^{1}LH$_3^+$, (b) = ^{1}LH$_2$, (c) = ^{1}LH$^-$, (d) = [Zn^{1}L], (e) = [Zn(^{1}L)$_2$]$^{2-}$, (f) = [Zn^{2}L^{1}L], and (g) = ^{1}L^{2-}.
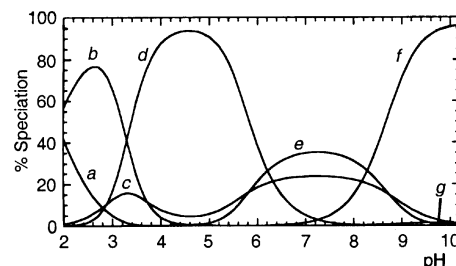
Figure 2. Speciation plot for a 50% aqueous ethanol (v/v) solution 4.99 × 10^{-3} mol dm^{-3} in [^{3}L]$_{total}$, 3.01 × 10^{-3} mol dm^{-3} in [Zn^{2+}]$_{total}$ and 1.04 × 10^{-5} in [^{1}LH$_n^{(n-2)+}$]$_{total}$ at 298.2 K and I = 0.10 (NaClO$_4$). Species are shown as percentages where 100% = [^{1}LH$_n^{(n-2)+}$]$_{total}$. (a) = ^{1}LH$_3^+$, (b) = ^{1}LH$_2$, (c) = ^{1}LH$^-$, (d) = [Zn^{1}L], (e) = [Zn^{1}L$_2$]$^{2-}$, (f) = [Zn^{3}L^{1}L], and (g) = ^{1}L^{2-}.

Two pK_as, assigned to dissociation of two protons from both [Zn^{5}LH^{1}LH]$^{2+}$ and [Zn^{7}LH^{1}LH]$^+$, were also determined as was a single pK_a attributed to dissociation of a proton from [Zn^{6}L^{1}LH]$^+$ (Table 2). The protonation sites cannot be assigned with certainty from these data alone. However, ^{1}L^{2-} and ^{1}LH$^-$ are the only fluorophore species likely to be coordinated by Zn^{2+} and therefore could bind a maximum of one proton in a protonated ternary complex, although ^{5}L could bind one proton, and ^{6}L and ^{7}L^{3-} could bind two protons and still act as bidentate ligands. The pK_a for the deprotonation of a coordinated hydroxy group in [Zn^{8}LH$_{-1}$^{1}L]$^-$ to give [Zn^{8}LH$_{-2}$^{1}L]$^{2-}$ was also determined (Table 2).

Speciation plots for the formation of [Zn^{2}L^{1}L] and [Zn^{3}L^{1}L], where [^{1}LH$_{(n=0-3)}^{(n-2)+}$]$_{total}$ is set to 100% and the percentage variations of all ^{1}L containing species with pH are shown in Figures 1 and 2 and in Figures S1−S5 provided as Supporting Information. Because of their much higher concentrations, the other Zn^{2+} and ^{2,3}LH$_{(n=0-3)}^{(3-n)+}$ containing species are not shown. It is seen (Figure 1) that [Zn^{2}L^{1}L] first appears at pH 4.2 and is the dominant species at pH 6.6 (70.1%), at which fluorescence determinations were made. The [Zn^{2}L^{1}L] rises to a maximum of 96.6% at pH 9.0 before decreasing in concentration coincident with ^{1}L^{2-} and [Zn^{2}L(OH)]$^+$ (whose protonated precursors have pK_as of 10.01 and 7.75, respectively) becoming the dominant free fluorophore and Zn^{2+} complex, respectively, as pH increases. In contrast, [Zn^{3}L^{1}L] only reaches < 1% at pH 6.6 (Figure 2) and a maximum of 95.6% at pH 10.0. The differences between the two systems illustrates the effects of ^{2}LH$_3^{3+}$ and ^{3}LH$_3^{3+}$ pK_as and [Zn^{2}L^{1}L] and [Zn^{3}L^{1}L] complexation constants on speciation. It is notable that largely because of the higher pK_{a1} and pK_{a2} of ^{3}LH$_3^{3+}$ and lesser K_1 of
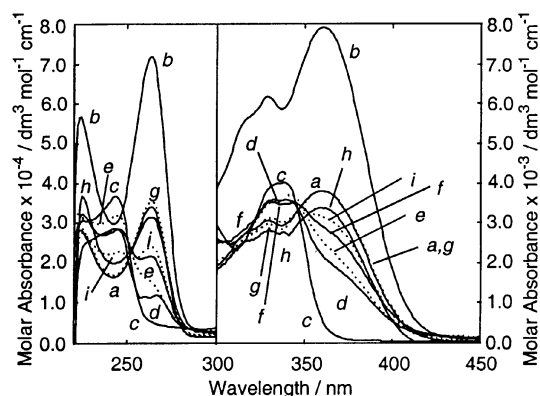
Figure 3. Absorbance spectra of (a) [Zn^{1}L] (pH 6.6), (b) [Zn(^{1}L)$_2$]$^{2-}$ (pH 6.6), (c) ^{1}LH$^-$ (pH 6.6), (d) [Zn^{2}L^{1}L] (pH 6.6), (e) [Zn^{3}L^{1}L] (pH 10.0, broken line), (f) [Zn^{4}L^{1}L] (pH 6.6), (g) [Zn^{5}L^{1}L] (pH 6.6 and 10.0, broken line), (h) [Zn^{5}LH^{1}L]$^+$ (pH 6.6), and (i) [Zn^{6}L^{1}L] (pH 6.6, broken line), in 50% aqueous ethanol (v/v) solution at 298.2 K. The pH of the solutions from which the spectra were derived is shown in brackets. The pH 6.6 solutions were 0.10 mol dm^{-3} in NaPIPES buffer, and the pH 10 solutions were 0.10 mol dm^{-3} in borate buffer.

[Zn^{3}L]$^{2+}$ by comparison with those of ^{2}LH$_3$$^{3+}$ and [Zn^{2}L]$^{2+}$, respectively, a substantial formation of [Zn(^{1}L)$_2$]$^{2-}$ occurs in the first system (Figure 2), whereas only a very small amount forms in the second. (The latter situation usually applies for the systems studied as is seen from the speciation plots in Figures S1–S5. The percent speciation refers to [Zn(^{1}L)$_2$]$^{2-}$ and should be multiplied by 2 for this species to obtain the percentage of ^{1}L^{2-} coordinated.) Because of such speciation variations with pH UV–vis and fluorescence determinations were made at either pH 6.6 or pH 10.0 or both, where the ternary complexes were at significant concentration.

In contrast to the above two systems, three ternary complexes: [Zn^{5}LH^{1}LH]$^{2+}$, [Zn^{5}LH^{1}L]$^+$, and [Zn^{5}L^{1}L] appear in the ^{5}L system where their proportions are 3.7%, 31.2%, and 62.2%, respectively, at pH 6.6, and that of [Zn^{5}L^{1}L] is 100% at pH 10.0. The [Zn^{4}L^{1}L] complex exists as 45.9% and 100% at pH 6.6 and 10.0, respectively, and [Zn^{6}L^{1}L] exists as 99.1% and 100% proportions at pH 6.6 and 10.0, respectively. The complexes, [Zn^{7}L^{1}LH]$^{2-}$ and [Zn^{7}L^{1}L]$^{3-}$ exist as 84.7% and 7.4%, respectively, at pH 6.6, and [Zn^{7}L^{1}L]$^{3-}$ exists as 97.3% at pH 10.0. Finally, [Zn^{8}LH$_{-1}$^{1}L]$^-$ exists as 15.3% at pH 6.0 and [Zn^{8}LH$_{-2}$^{1}L]$^{2-}$ as 92.5% at pH 10.0. (These species percentages apply at the reagent concentrations given in the captions to Figures 1 and 2 and Figures S1–S5 and for the fluorescence determinations in the Experimental Section, and their variations reflect the variations in K_1–K_5 and the associated pK_as controlling the competing equilibria.)

Solution UV–Vis Absorbance Studies. Because of the substantial variation of speciation with pH, UV–vis spectra were run at pH 6.6 for the ^{2}L, ^{4}L, ^{5}L, ^{6}L, ^{7}L^{3-}, and ^{8}L systems, and at pH 10 for the ^{3}L, ^{5}L, ^{7}L^{3-}, and ^{8}L systems. The UV–vis spectra of the individual species in solution were derived from solution spectra on the basis of the concentrations of each ternary Zn^{2+} complex calculated from the data in Tables 1 and 2, and reported UV–vis spectra of ^{1}LH$^-$, [Zn^{1}L], and [Zn(^{1}L)$_2$]$^{2-}$.[3] The derived UV–vis spectra are shown in Figures 3 and 4, and the molar absorbances at the spectral maxima are given in Table 3. In some cases the noise level of the derived spectrum is significant as the complex of interest is not the only absorbing species contributing to the observed total absorbance. (Significant noise levels similarly arise in some of the derived
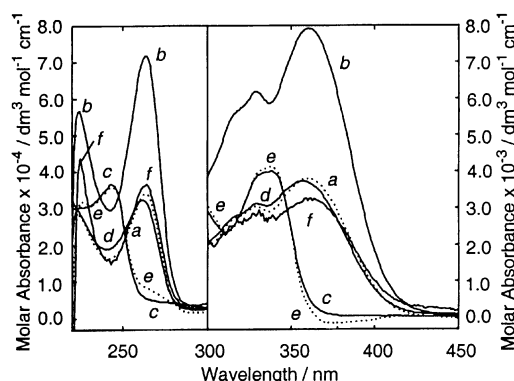

Figure 4. (a) [Zn^{1}L] (pH 6.6, broken line), (b) [Zn(^{1}L)$_2$]$^{2-}$ (pH 6.6) (c) ^{1}LH$^-$ (pH 6.6), (d) [Zn^{7}L^{1}L]$^{3-}$ (pH 10.0), (e) [Zn^{7}L^{1}LH]$^{2-}$ (pH 6.6, broken line), and (f) [Zn^{8}LH$_{-1}$^{1}L]$^-$ (pH 6.6) in 50% aqueous ethanol (v/v) solution at 298.2 K. The pH of the solutions from which the spectra were derived is shown in brackets. The pH 6.6 solutions were 0.10 mol dm^{-3} in NaPIPES buffer, and the pH 10 solutions were 0.10 mol dm^{-3} in borate buffer.

fluorescence spectra as seen in Figure 5.) The accumulation of errors arising from deriving system speciation potentiometrically and applying it in the derivation of species spectra leads to the absorbance of [Zn^{7}L^{1}LH]$^{2-}$ becoming negative between 360 and 400 nm were absorbance is low (Figure 4). The molar absorbance of [Zn(^{1}L)$_2$]$^{2-}$ is approximately twice that of the other complexes because of the presence of two ^{1}L^{2-}.[3] The reagent concentrations of the solutions studied appear in the Experimental Section.

Of the spectra of the eight ternary complexes, the wavelength maxima of those of [Zn^{5}L^{1}L], [Zn^{5}LH^{1}L]$^+$, [Zn^{7}L^{1}L]$^{3-}$, and [Zn^{8}LH$_{-1}$^{1}L]$^{3-}$ are similar to those of [Zn^{1}L] which may indicate that the coordination environment of ^{1}L^{2-} is similar in the five complexes. The similarity of the spectrum of [Zn^{5}LH^{1}L]$^+$ to that of [Zn^{1}L] is consistent with ^{5}L being protonated rather than ^{1}L^{2-} as expected from the pK_as of their monoprotonated forms in the free state, 11.69 and 10.01, respectively. In contrast, the wavelength maxima in the spectrum of [Zn^{7}L^{1}LH]$^{2-}$ closely resemble those of ^{1}LH$^-$ consistent with protonation being on ^{1}L^{2-} as anticipated from the pK_a of free ^{7}LH^{2-} being 9.02, whereas that of ^{1}LH$^-$ is 10.1. The spectra of the ternary complexes of ^{2}L, ^{3}L, ^{4}L, and ^{6}L show characteristics intermediate between those of ^{1}LH$^-$ and [Zn^{1}L] consistent with these polyamines modifying the environment of coordinated ^{1}L^{2-} by comparison with that in [Zn^{1}L].

Solution Fluorescence Studies. Fluorescence spectra were run at pH 6.6 for the ^{2}L, ^{4}L, ^{5}L, ^{6}L, ^{7}L^{3-}, and ^{8}L systems, and at pH 10.0 for the ^{3}L, ^{5}L, and ^{7}L^{3-} systems, to accommodate the substantial variation of speciation with pH. The fluorescence spectra for individual complexes were derived from the fluorescence spectra of the solutions studied on the basis of the reported fluorescence spectra of [Zn^{1}L] and [Zn(^{1}L)$_2$]$^{2-}$ and the concentrations of each Zn^{2+} ternary complex of ^{1}L^{2-} in solution as determined from the data in Tables 1 and 2. In all cases, excitation was at 358 nm, and the fluorescence spectrum of each species was divided by the molar absorbance at 358 nm to produce the standardized spectra in Figure 5. (Because of its small molar absorbance at 358 nm, the derived fluorescence spectrum of [Zn^{7}L^{1}LH]$^{2-}$ may be subject to considerable error and is not considered. It is probable that the ^{1}LH$^-$ ligand will be at most weakly fluorescent.) The molar absorbances at 358 nm, emission λ_{max}, and relative emission intensities at λ_{max}, and quantum yields, ϕ, appear in Table 4.

Table 3. Derived UV–Vis Spectra of Ternary Zn^{2+} Complexes in 50% Aqueous Ethanol (v/v) at 298.2 K

species	wavelength at maximum in nm (and molar absorbance in dm^3 mol^{-1} cm^{-1})						
^{1}LH^{-} [a,b]	225	243	336				
	(3.06×10^4)	(3.66×10^4)	(3.99×10^3)				
[Zn^{1}L][a,b]	224	263	315[b]	330	361		
	(2.82×10^4)	(3.40×10^4)	(2.68×10^3)	(3.07×10^3)	(3.81×10^3)		
[Zn(^{1}L)$_2$]$^{2-}$ [a,b]	224	263	315[c]	329	361		
	(5.67×10^4)	(7.18×10^4)	(5.52×10^3)	(6.19×10^3)	(7.92×10^3)		
[Zn^{2}L^{1}L][b]	225	243	260	267	333	339	368[c]
	(2.39×10^4)	(2.87×10^4)	(1.16×10^4)	(1.20×10^4)	(3.58×10^3)	(3.58×10^3)	(1.88×10^3)
[Zn^{3}L^{1}L] [d]	244	263[c]	331	341	368[b]		
	(3.20×10^4)	(1.54×10^4)	(3.53×10^3)	(3.71×10^3)	(2.65×10^3)		
[Zn^{4}L^{1}L] [b]	225	245	263	315[c]	330	345	368
	(3.25×10^4)	(2.83×10^4)	(2.17×10^4)	(2.82×10^3)	(3.52×10^3)	(3.51×10^3)	(2.65×10^3)
[Zn^{5}L^{1}L] [b,d]	223	264	315[c]	329	361		
	(2.97×10^4)	(2.59×10^3)	(2.59×10^3)	(2.98×10^3)	(3.81×10^3)		
[Zn^{5}L^{1}LH]$^{+}$ [b]	224	263	315[b]	330	361		
	(3.69×10^4)	(3.15×10^4)	(2.48×10^3)	(2.86×10^3)	(3.39×10^3)		
[Zn^{6}L^{1}L] [b]	225	245	263	315[c]	330	351	
	(2.76×10^4)	(2.27×10^4)	(2.40×10^4)	(2.58×10^3)	(3.12×10^3)	(3.24×10^3)	
[Zn^{7}L^{1}L]$^{3-}$ [d]	261	315[c]	330	356			
	(3.22×10^4)	(2.73×10^3)	(3.12×10^3)	(3.73×10^3)			
[Zn^{7}L^{1}LH]$^{2-}$ [b]	225	244	267[c]	336			
	(3.18×10^4)	(3.59×10^4)	(7.44×10^3)	(4.11×10^3)			
[Zn^{8}LH$_{-1}{}^1$L]$^{-}$ [b]	225	244	267[c]	336			
	(3.18×10^4)	(3.59×10^4)	(7.44×10^3)	(4.11×10^3)			

[a] Ref 3. [b] pH 6.6, 0.10 mol dm^{-3} NaPIPES buffer. [c] Shoulder. [d] pH 10.0, 0.10 mol dm^{-3} borate buffer.
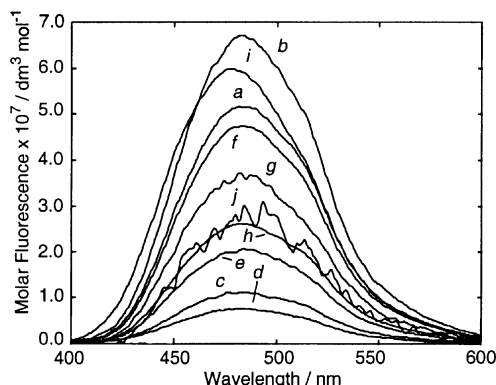
Figure 5. Fluorescence spectra of (a) [Zn^{1}L] (pH 6.6), (b) [Zn(^{1}L)$_2$]$^{2-}$ (pH 6.6), (c) [Zn^{2}L^{1}L] (pH 6.6), (d) [Zn^{3}L^{1}L] (pH 10.0), (e) [Zn^{4}L^{1}L] (pH 6.6), (f) [Zn^{5}L^{1}L] (pH 6.6 and 10.0), (g) [Zn^{5}LH^{1}L]$^+$ (pH 6.6), (h) [Zn^{6}L^{1}L] (pH 6.6), (i) [Zn^{7}L^{1}L]$^{3-}$ (pH 10.0), and (j) [Zn^{8}LH$_{-1}{}^1$L]$^-$ (pH 6.6) in 50% aqueous ethanol (v/v) solution at 298.2 K. The pH of the solutions from which the spectra were derived is shown in brackets. The pH 6.6 solutions were 0.10 mol dm^{-3} in NaPIPES buffer, and the pH 10 solutions were 0.10 mol dm^{-3} in borate buffer.

There is a 5-fold ϕ variation for the complexes in Table 4 consistent with a significant effect of the coordination environment on the fluorescence of ^{1}L^{2-}. This is attributable to differing excitation and quenching efficiencies in the range of complexes studied. The ϕ of [Zn^{5}L^{1}L], [Zn^{5}LH^{1}L]$^+$, and [Zn^{7}L^{1}L]$^{3-}$ most closely approach that of [Zn^{1}L] consistent with the coordination environments of ^{1}L^{2-} being the most similar as was also deduced from the UV-vis spectra.

The absence of detectable fluorescence for ^{1}LH$^-$ contrasts with the significant fluorescence observed for ^{1}L^{2-} in [Zn^{1}L], [Zn(^{1}L)$_2$]$^{2-}$ and the ternary complexes. This is probably attributable to a combination of increased delocalization of π electron density in coordinated ^{1}L^{2-} coincident with the sulfonamide nitrogen lone pair coordinating with Zn^{2+}, and a restriction of vibrational and internal rotational modes of coordinated ^{1}L^{2-} resulting in a decrease in quenching efficiency by comparison with that of ^{1}LH$^-$. This deduction is based on the sulfonamide

Table 4. Fluorescence Parameters for Ternary Zn^{2+} Complexes in 50% Aqueous Ethanol (v/v) at 298.2 K

complex	molar absorbance 358 nm	emission λ_{max} (nm)	relative emission at λ_{max}	ϕ (400–600 nm)
[Zn^{1}L][a,b]	3.79×10^3	485	5.16×10^7	0.21
[Zn^{1}L$_2$]$^{2-}$ [a,b]	7.88×10^3	484	6.72×10^7	0.13
[Zn^{2}L^{1}L][b]	2.29×10^3	484	1.11×10^7	0.08
[Zn^{3}L^{1}L][c]	2.64×10^3	484	7.62×10^6	0.05
[Zn^{4}L^{1}L][b]	2.97×10^3	484	2.05×10^7	0.10
[Zn^{5}L^{1}L][b,c]	3.79×10^3	484	4.74×10^7	0.20
[Zn^{5}LH^{1}L]$^{+}$ [b,c]	3.38×10^3	484	3.68×10^7	0.17
[Zn^{6}L^{1}L][b]	3.19×10^3	484	2.61×10^7	0.13
[Zn^{7}L^{1}L]$^{3-}$ [b,c]	3.73×10^3	478	5.98×10^7	0.25
[Zn^{8}LH$_{-1}{}^1$L]$^{-}$ [b]	$\sim 3.25 \times 10^3$	~ 492	$\sim 2.78 \times 10^7$	~ 0.14

[a] Ref 3. [b] pH 6.6, 0.10 mol dm^{-3} NaPIPES buffer. [c] pH 10.0, 0.10 mol dm^{-3} borate buffer.

nitrogen of the closely related 6-methoxy-(8-*p*-toluenesulfonamido)quinoline bidentate ligand approaching trigonal planar stereochemistry in its tetrahedral bis complex of Zn^{2+}.[12]

Conclusions

Zinquin A anion, ^{1}L^{2-}, fluoresces in the ternary [Zn${}^{2-6}$L^{1}L], [Zn^{5}LH^{1}L]$^+$, [Zn^{7}L^{1}L]$^{3-}$, and [Zn^{8}LH$_{-1}{}^1$L]$^-$ complexes. The fluorescence λ_{max} varies slightly and ϕ varies 5-fold as the ^{1}L^{2-} coordination environment changes with the nature of the ligands ${}^{2-6,8}$L and ^{7}L^{3-}. This suggests that most of the fluorescence of ^{1}L^{2-} observed in intracellular studies arises from the coordination of ^{1}L^{2-} by Zn^{2+} that also coordinates a protein or other bioligand. Such coordination of ^{1}L^{2-} may arise either when two waters coordinated to Zn^{2+} in a protein complex are substituted by bidentate ^{1}L^{2-}, or Zn^{2+} increases its coordination number from <6 to 6 to accommodate bidentate coordination of ^{1}L^{2-}, or when ^{1}L^{2-} displaces weakly coordinated protein donor groups. These findings suggest that assessments of intracellular Zn^{2+} levels through Zn^{2+} specific fluorophores are likely to be semiquantitative and that different Zn^{2+} specific fluorophores probably detect different ensembles of biological Zn^{2+} species.

Experimental Section

Materials. [2-Methyl-8-(*p*-toluenesulfonamido)-6-quinolyloxy]acetic acid was prepared as described elsewhere.[2] Ligands **2L**, **4L** and **5L** were prepared as reported in the literature.[13] Ligand **3L** (Strem), Na_3**7L**H_{-3} (Fluka), $Zn(ClO_4)_2$ (Fluka) and $NaClO_4$ (Fluka) were twice recrystallized from water, and dried to constant weight over P_2O_5 under vacuum prior to use. (**CAUTION**. Perchlorate salts can be explosive under anhydrous conditions and should be handled with care.) After being recrystallized from water, **6L**(HCl)$_4$ (Aldrich) was dissolved in water, neutralized with NaOH, and **6L** was extracted into chloroform that was removed under vacuum and dried to constant weight over P_2O_5 under vacuum. Sodium piperazine-*N*,*N*′-bis(2-ethanesulfonate), NaPIPES (Cal Biochem), di-sodiumethylenediaminetetraacetic acid, Na_2edtaH$_2$ (Ajax), boric acid (Ajax), NaOH solution (Convol, BDH), $HClO_4$ (BDH Analar) and **8L** (BDH) were used as received. Deionized water was further purified using a MilliQ-Reagent system to produce water with a resistance of >15 MΩ cm. Analytical grade ethanol distilled from CaO was used in all solution preparations. Zinc perchlorate, $HClO_4$ and NaOH (all with *I* adjusted to 0.10 mol dm^{-3} with $NaClO_4$) titration solutions were prepared in 50% aqueous ethanol by volume under nitrogen and were standardized by conventional methods. Solutions for spectroscopic study were either buffered with 0.10 mol dm^{-3} NaPIPES buffer adjusted to pH 6.6 with NaOH and $HClO_4$, or with 0.10 mol dm^{-3} borate buffer adjusted to pH 10.0 with NaOH and $HClO_4$.

Potentiometric Titrations. Potentiometric titrations were carried out using a Metrohm E665 Dosimat autoburet interfaced to a Laser XT/3−8086 PC in conjunction with an Orion SA720 potentiometer and an Orion Ross Sureflow combination electrode. The electrode was calibrated using standard buffer solutions and no corrections were made to pH values determined in the 50% aqueous ethanol (v/v) solutions. Titration solutions were thermostated at 298.2 ± 0.05 K in a water-jacketed vessel that was closed apart from a small vent for the nitrogen stream that was bubbled through the magnetically stirred titration solutions to exclude atmospheric carbon dioxide. The instrumentation was calibrated by titration of 0.100 mol dm^{-3} NaOH (1.00 cm^3) from the autoburet against 0.004 mol dm^{-3} $HClO_4$ (10.00 cm^3). Protonation constants for the ligands, **2L** − **6L** and **7L**$^{3-}$, were determined by titration of 10.00 cm^3 solutions (2.00−2.94) × 10^{-3} mol dm^{-3} in the ligand and (2.00−4.01) × 10^{-3} mol dm^{-3} in $HClO_4$ (such that the acid concentration was at least one mole in excess of that required to completely protonate the ligand) with 0.100 mol dm^{-3} NaOH. The stepwise complexation constants for the binary and bis Zn^{2+} complexes were carried out at the same concentrations of ligand and $HClO_4$ in the presence of Zn^{2+} in a series of two titrations; the first were characterized by [Zn^{2+}]$_{total}$/[ligand]$_{total}$ = 0.5 and the second by [Zn^{2+}]$_{total}$/[ligand]$_{total}$ = 1.0. The stepwise complexation constants for the ternary Zn^{2+} complexes were determined from titration of solutions (3.91−4.13) × 10^{-3} mol dm^{-3} in the ligand, **2L** − **6L** and **7L**$^{3-}$, and (8.01−10.0) × 10^{-3} mol dm^{-3} in $HClO_4$. In all solutions [Zn^{2+}]$_{total}$ and [**1L**$^{2-}$]$_{total}$ = 0.5 × [**2-6L**, **7L**$^{3-}$, and **8L**]$_{total}$. All titrations were carried out in triplicate at least. Generally, the pH titration range was 2.0 to 10.5 except for titrations of **4L** where precipitation of [Zn**4L**(OH)]$^+$ commencing at pH 8.5 precluded titration to a higher pH. The pK_as and stepwise Zn^{2+} complexation constants were determined using program SUPERQUAD[14] and appear in Tables 1 and 2.

UV−Vis Spectroscopy. UV−vis spectrophotometric determinations were performed on solutions thermostated at 298.2 ± 0.03 K in stoppered 1 cm path length silica cells using a Cary 2200 spectrophotometer. For determinations in the 220−300 nm region at pH 6.6 all solutions were 1.97 × 10^{-5} mol dm^{-3} in [**1L**$^{2-}$]$_{total}$, and the other reagent concentrations (mol dm^{-3}) in the duplicate five solutions studied were

[Zn^{2+}]$_{total}$ = 9.08 × 10^{-5} and [**2L**]$_{total}$ = 2.07 × 10^{-4}; [Zn^{2+}]$_{total}$ = 9.08 × 10^{-5} and [**4L**]$_{total}$ = 2.01 × 10^{-4}; [Zn^{2+}]$_{total}$ = 8.08 × 10^{-5} and [**5L**]$_{total}$ = 4.92 × 10^{-3}; [Zn^{2+}]$_{total}$ = 9.08 × 10^{-5} and [**6L**]$_{total}$ = 2.00 × 10^{-3}; [Zn^{2+}]$_{total}$ = 3.01 × 10^{-3} and [**7L**$^{3-}$]$_{total}$ = 4.98 × 10^{-3}; and [Zn^{2+}]$_{total}$ = 3.01 × 10^{-3} and [**8L**]$_{total}$ = 5.20 × 10^{-3}. For determinations in the 220−300 nm region at pH 10.0 [**1L**]$_{total}$ = 2.07 × 10^{-5} mol dm^{-3} and the other reagent concentrations (mol dm^{-3}) in the duplicate three solutions studied were [Zn^{2+}]$_{total}$ = 3.01 × 10^{-3} and [**3L**]$_{total}$ = 4.99 × 10^{-3}; [Zn^{2+}]$_{total}$ = 4.51 × 10^{-4} and [**5L**]$_{total}$ = 5.05 × 10^{-3}; and [Zn^{2+}]$_{total}$ = 3.01 × 10^{-3} and [**7L**$^{3-}$]$_{total}$ = 5.03 × 10^{-3}. For determinations in the 300−450 nm region at pH 6.6 all solutions were 9.84 × 10^{-5} mol dm^{-3} in [**1L**$^{2-}$]$_{total}$, and the other reagent concentrations (mol dm^{-3}) in the duplicate six solutions studied were [Zn^{2+}]$_{total}$ = 4.51 × 10^{-4} and [**2L**]$_{total}$ = 1.03 × 10^{-3}; [Zn^{2+}]$_{total}$ = 4.51 × 10^{-4} and [**4L**]$_{total}$ = 1.01 × 10^{-3}; [Zn^{2+}]$_{total}$ = 4.51 × 10^{-4} and [**5L**]$_{total}$ = 5.00 × 10^{-3}; [Zn^{2+}]$_{total}$ = 4.51 × 10^{-4} and [**6L**]$_{total}$ = 1.00 × 10^{-3}; [Zn^{2+}]$_{total}$ = 4.01 × 10^{-4} and [**7L**$^{3-}$]$_{total}$ = 4.98 × 10^{-3}; and [Zn^{2+}]$_{total}$ = 4.51 × 10^{-4} and [**8L**]$_{total}$ = 5.20 × 10^{-3}. For determinations in the 300−450 nm region at pH 10.0 [**1L**]$_{total}$ = 1.04 × 10^{-5} mol dm^{-3} and the other reagent concentrations (mol dm^{-3}) in the duplicate three solutions studied were [Zn^{2+}]$_{total}$ = 3.01 × 10^{-3} and [**3L**]$_{total}$ = 4.99 × 10^{-3}; [Zn^{2+}]$_{total}$ = 4.51 × 10^{-4} and [**5L**]$_{total}$ = 5.05 × 10^{-3}; and [Zn^{2+}]$_{total}$ = 3.01 × 10^{-3} and [**7L**$^{3-}$]$_{total}$ = 5.03 × 10^{-3}. The spectra of the ternary complexes were obtained by fitting the observed absorbances to the reported spectra of [Zn**1L**] and [Zn(**1L**)$_2$]$^{2-}$ the appropriate potentiometrically determined stepwise stability constants through a nonlinear least squares regression analysis based on the Method 5 of Pitha and Jones[15] using an AcerPower 466d computer. The fluorescence spectra were similarly derived from the fluorescence of the solutions discussed below.

Fluorimetry. Fluorimetric determinations were performed on solutions thermostated at 298.2 ± 0.03 K in stoppered 1 cm path length silica cells using a Perkin Elmer LS50B fluorimeter. For determinations at pH 6.6 all solutions were 9.84 × 10^{-6} mol dm^{-3} in [**1L**$^{2-}$]$_{total}$, and the other reagent concentrations (mol dm^{-3}) in the six solutions studied were [Zn^{2+}]$_{total}$ = 4.58 × 10^{-5} and [**2L**]$_{total}$ = 1.03 × 10^{-4}; [Zn^{2+}]$_{total}$ = 4.58 × 10^{-5} and [**4L**]$_{total}$ = 1.01 × 10^{-4}; [Zn^{2+}]$_{total}$ = 4.08 × 10^{-5} and [**5L**]$_{total}$ = 4.99 × 10^{-3}; [Zn^{2+}]$_{total}$ = 4.58 × 10^{-5} and [**6L**]$_{total}$ = 1.00 × 10^{-4}; and [Zn^{2+}]$_{total}$ = 3.50 × 10^{-3} and [**7L**$^{3-}$]$_{total}$ = 4.98 × 10^{-3}; [Zn^{2+}]$_{total}$ = 1.50 × 10^{-3} and [**8L**]$_{total}$ = 5.20 × 10^{-3}. For determinations at pH 10.0 [**1L**$^{2-}$]$_{total}$ = 1.04 × 10^{-5} mol dm^{-3} and the other reagent concentrations (mol dm^{-3}) in the three solutions studied were [Zn^{2+}]$_{total}$ = 3.01 × 10^{-3} and [**3L**]$_{total}$ = 4.99 × 10^{-3}; [Zn^{2+}]$_{total}$ = 4.51 × 10^{-4} and [**5L**]$_{total}$ = 5.05 × 10^{-3}; and [Zn^{2+}]$_{total}$ = 3.01 × 10^{-3} and [**7L**$^{3-}$]$_{total}$ = 5.03 × 10^{-3}. Quantum yields were determined using quinine as the reference fluorophore and were not corrected for solvent refractive index.[16]

Adventitious Zn^{2+}. Zinc(II) is environmentally ubiquitous and is present as a very low level impurity in some high-grade commercial chemicals as shown by our atomic absorption measurements. This is a problem when working with low experimental Zn^{2+} concentrations in fluorescence studies.[3] Either, all components of a solution may be further purified at the risk of introducing other impurities, or the Zn^{2+} impurity level may be determined in situ. The second approach was adopted and the [Zn^{2+}]$_{adventitious}$ = 8.0 × 10^{-7} mol dm^{-3} was the highest level found in buffer solutions made up in the $NaClO_4$ in supporting electrolyte in the absence of **1L**H$_2$, **2-6L**, and **7L**$^{3-}$. We found [Zn^{2+}]$_{adventitious}$ to be below detection levels in solutions of the ligand over the experimental ranges studied. Thus, [Zn^{2+}]$_{adventitious}$ made less than a 2% contribution to [Zn^{2+}]$_{total}$ in the most dilute solutions used in the fluorescence studies, and could be neglected in potentiometric and spectrophotometric studies where [Zn^{2+}]$_{total}$ was much greater.

(13) Richman, J. E.; Atkins, T. J. *J. Am. Chem. Soc.* **1974**, *96*, 2268. Searle, G. H.; Geue, R. J. *Aust. J. Chem.* **1984**, *37*, 659. Briellmann, M.; Kaderli, S.; Meyer, C. J.; Zuberbühler, A. D. *Helv. Chim. Acta* **1987**, *70*, 680.
(14) Gans, P.; Sabatini, A.; Vacca, A. *J. Chem. Soc.*, *Dalton Trans.* **1985**, 1195.

(15) Pitha, J.; Jones, R. N. *Can. J. Chem.* **1966**, *44*, 3031
(16) Melhuish, W. H. *J. Phys. Chem.* **1961**, *65*, 229.

Supporting Information Available: Speciation plots for the compounds in this work (Figures S1−S5). This material is available free of charge via the Internet at http://pubs.acs.org.

JA020685Y