A preparative, spectroscopic and equilibrium study of some phenyl-2-thiazoline fluorophores for aluminium(III) detection

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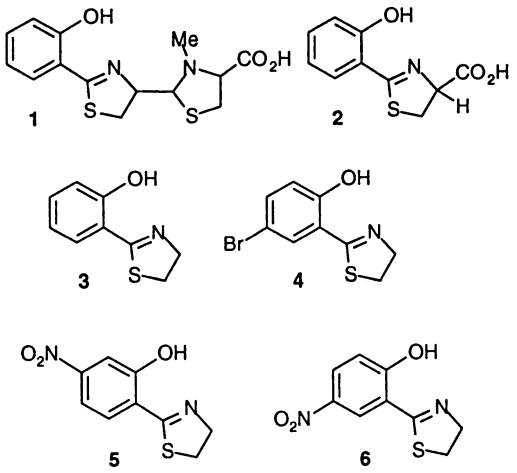
Received (in Montpellier, France) 8th March 2000, Accepted 12th April 2000
Published on the Web 6th June 2000

As part of a study of fluorophores for selective Al^{3+} detection and of potential use in the study of intracellular Al^{3+}, the preparative, UV–visible and fluorescence spectroscopic, and Al^{3+} coordination characteristics of five ligands containing the conjugated phenolic substituted thiazoline chromophore of the natural siderophore, pyochelin, 2-[2-(2-hydroxyphenyl)-4,5-dihydro-1,3-thiazol-4-yl]-3-methyl-1,3-thiazolane-4-carboxylic acid) **1**, are reported. The five ligands are 2-(2-hydroxyphenyl)-4,5-dihydro-1,3-thiazole-4-carboxylic acid, **2**, 2-(4,5-dihydro-1,3-thiazol-2-yl)phenol, **3**, 4-bromo-2-(4,5-dihydro-1,3-thiazol-2-yl)phenol, **4**, 2-(4,5-dihydro-1,3-thiazol-2-yl)-5-nitrophenol, **5**, and 2-(4,5-dihydro-1,3-thiazol-2-yl)-4-nitrophenol, **6**. All form stable binary Al^{3+} complexes in 75% methanol and 25% water in the case of **2**, and 73.2% methanol, 24.4% water and 2.4% *N*,*N*-dimethylformamide in the case of **3**–**6**, and exhibit substantial UV–visible absorbance changes on coordination. However, while **2**, **3** and **4** fluoresce strongly when coordinated to Al^{3+}, **5** and **6** do not, probably because their fluorescence is quenched through a twisted intramolecular charge transfer process. All five ligands show selectivity in complexing for Al^{3+} over other metal ions and, in the case of **2**, **3** and **4**, represent an initial stage in the development of Al^{3+} specific fluorophores for biological use.

Aluminium is the third most abundant element in the earth's crust, but until recently its biological availability has been low because it is predominantly bound as Al^{3+} in aluminosilicates and $[Al(OH)_3]$, which have low aqueous solubilities.[1–4] However, the advent of acid rain in industrial regions has lowered soil pH and released Al^{3+} into ground water with a deleterious effect on forest and aquatic systems. The use of aluminium utensils and containers for food and beverage handling,[5,6] and Al^{3+} containing pharmaceuticals[2] have also increased Al^{3+} bioavailability. Although at the physiological pH of 7.4 the low solubility of $Al(OH)_3$ limits uncomplexed $[Al^{3+}]$ to $\leqslant 10^{-11}$ mol dm^{-3} in plasma, its concentration as citrate, transferrin and adenosine and guanosine triphosphate complexes may be substantially higher.[7,8] Consequently, there has been increasing interest in the transport of Al^{3+} at the cellular level,[9,10] and a need for the development of a sensitive non-destructive detection method for intracellular Al^{3+}. Fluorescent emission from Ca^{2+} and Zn^{2+} specific fluorophores has proved to be a particularly sensitive method for determining the *in situ* variations of the intracellular levels of these ions.[11–15] This study represents an initial phase in the development of Al^{3+} specific fluorophores for intracellular use.

Pyochelin, 2-[2-(2-hydroxyphenyl)-4,5-dihydro-1,3-thiazol-4-yl]-3-methyl-1,3-thiazolane-4-carboxylic acid, **1**, isolated from *Psedomonas aeruginosa*, is a siderophore that forms a red complex with Fe^{3+}.[16] Because of its propensity to coordinate Fe^{3+}, pyochelin is likely to coordinate another hard Lewis acid, Al^{3+}, and thereby provide a starting point for the development of Al^{3+} specific fluorophores. The pyochelin chromophore is the conjugated phenolic substituted thiazoline moiety, which exhibits absorbance maxima at 218 (10 500), 248 (10 500) and 310 nm (4200 dm^3 mol^{-1} cm^{-1}) where the figures in parentheses are molar absorbances.[16] Accordingly, this study is predominantly concerned with the coordination by Al^{3+} of ligands containing the pyochelin chromophore and the effect of such coordination on the UV–visible absorbance and fluorescence of these ligands. To act as a fluorophore for the detection of Al^{3+} it is necessary that the excited state of the pyochelin chromophore is sufficiently stabilised through Al^{3+} coordination to generate significant fluorescence. The ligands studied are 2-(2-hydroxyphenyl)-4,5-dihydro-1,3-thiazole-4-carboxylic acid, **2**, 2-(4,5-dihydro-1,3-thiazol-2-yl) phenol, **3**,4-bromo-2-(4,5-dihydro-1,3-thiazol-2-yl)phenol, **4**, 2-(4,5dihydro-1,3-thiazol-2-yl)-5-nitrophenol, **5**, and 2-(4,5-dihydro-1,3-thiazol-2-yl)-4-nitrophenol, **6**. This appears to be one of the first systematic studies of complexation by Al^{3+} specific fluorophores.



Results and discussion

Ligand synthesis and characterisation

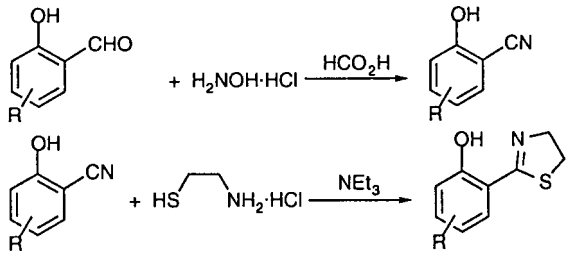
Reported synthetic methods were used to obtain **2** and **3** in good yield.[17,18] The general method for the preparation of the

thiazolines **4**–**6** is shown in Scheme 1. Conversion of a benzaldehyde derivative to the corresponding benzonitrile derivative by refluxing with hydroxylamine hydrochloride in formic acid was followed by formation of the thiazoline ring through reaction of the benzonitrile derivative with 2-aminoethanethiol hydrochloride in the presence of triethylamine. To obtain sufficient solubility of the ligands for the studies discussed below it was necessary to use a 75% methanol–25% aqueous (v : v) solvent mixture to make up solutions. Because of their slow dissolution rates, stock solutions of **3**–**6** were prepared in *N*,*N*-dimethylformamide (DMF) so that their final solutions were 73.2% methanol, 24.4% water and 2.4% DMF by volume. Under the conditions of this study **2** exhibits two pK_as and **3**–**6** one pK_a each as listed in Table 1. Ligands **2**–**6** show strong absorbance in the UV–visible region but none shows significant fluorescence in the free state.

It is usually necessary for biological probe molecules to possess significant lipophilic character so that they can traverse the cell membrane. As a consequence, such molecules may possess low aqueous solubilities and are consequently studied in aqueous solvent mixtures to increase their concentrations to those required for pK_a determinations and UV–visible spectroscopic studies as is the case in this work. A similar situation prevailed for our Zn^{2+} specific fluorophore, 2-methyl-8-(toluene-*p*-sulfonamido)-6-quinolyloxyacetic acid,[15] which has been successfully used in the study of intracellular Zn^{2+}.[13,14]

UV–visible and fluorescence equilibrium studies

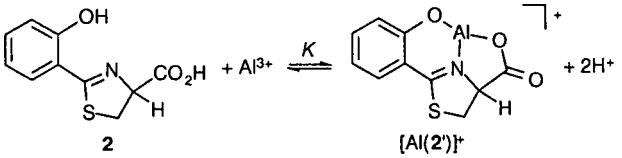
Hydrated Al^{3+} is strongly acidic and precipitates as $[Al(OH)_3]$ at pH $\geqslant$ 5. This precluded the use of pH-titrimetric methods use to determine the pK_as of **2**–**6** for the determination of Al^{3+} coordination of these ligands. However, it was both convenient and necessary to study the coordination of ligands **2**–**6** by UV–visible and fluorimetic methods to assess their suitability as Al^{3+} specific fluorophores. Most available buffers either complex Al^{3+}, or cause it to precipitate as a salt, or both, under the conditions of this study. Thus, the coordination of **2** was studied in 10^{-3} mol dm^{-3} $HClO_4$ solutions to maintain a constant $[H^+]$. However, **3**–**6** do not complex Al^{3+} at this high $[H^+]$ and accordingly their coordination was studied in the absence of added acid. These observations are consistent with Al^{3+} competing with H^+ for coordination sites in the dianion of **2**, **2′**, which probably acts as a tridentate ligand while the monoanions of **3**–**6**, **3′**–**6′**, probably act as bidentate ligands as shown in Schemes 2 and 3, respectively.



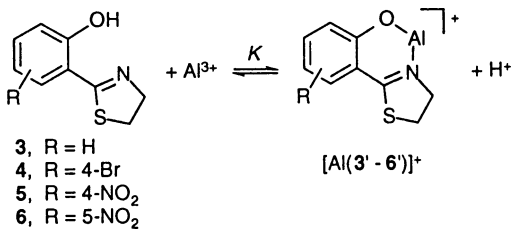
Scheme 1

Ligand 2. The variation of the UV-visible spectrum of **2** with $[Al^{3+}]_{total}$ is shown in Fig. 1, from which it is seen that three isosbestic points (259, 279 and 328 nm) exist consistent with the chromophore experiencing two dominant environments. Fitting of an algorithm for 1 : 1 complexation to the absorbance data in the ranges 290–315 nm and 335–375 nm gave a formation constant $K = 2390 \pm 20$ dm^3 mol^{-1} for $[Al(\mathbf{2}')]^+$ and a derived spectrum of coordinated **2′** with maxima at 270 (14 900) and 350 nm (6600 dm^3 mol^{-1} cm^{-1}), where the molar absorbances appear in parentheses. Under the same conditions, the spectrum of free **2** is characterised by maxima at 258 (9800), 275 (7700) and 311 (5600) and a shoulder at 350 nm (2600 dm^3 mol^{-1} cm^{-1}). The fitting of algorithms incorporating the additional formation of either 1 : 2 or 2 : 1 complexes to these data showed no evidence for their formation in either this system or in the other systems considered below.

The isosbestic point at 328 nm was chosen as the excitation wavelength for the fluorescence study of the coordination of **2** by Al^{3+}. The concentration of **2** was held constant at 3.80×10^{-7} mol dm^{-3} to optimise the fluorescence measurements over the range 400–500 nm in the presence of Al^{3+}. The increase in fluorescence of **2** with $[Al^{3+}]_{total}$ is shown in Fig. 2, from which it is seen that the broad fluorescence band shows a systematic increase in intensity with increase in $[Al^{3+}]_{total}$. When an algorithm for 1 : 1 complexation is fitted to these



Scheme 2



Scheme 3

Table 1 Ligand **2**–**6** pK_as and stability constants (K) of the 1 : 1 complexes of their conjugate bases **2′**–**6′** with Al^{3+} at 298.2 K

Ligand[a]	pK_a	K/dm^3 mol^{-1} [b] (UV–visible)	K/dm^3 mol^{-1} [b] (fluorescence)
2/**2′**	4.62 ± 0.01[c] 11.98 ± 0.01[c]	(2.39 ± 0.02) × 10^3 [d]	(1.83 ± 0.01) × 10^3 [d]
3/**3′**	>12.0	(5.57 ± 0.12) × 10^3	(3.19 ± 0.04) × 10^3
4/**4′**	11.12 ± 0.01	(7.08 ± 0.11) × 10^3	(3.89 ± 0.03) × 10^3
5/**5′**	9.81 ± 0.01	(1.20 ± 0.04) × 10^4	
6/**6′**	8.38 ± 0.01	(4.48 ± 0.20) × 10^4	

[a] For **2** all solutions were 75% methanol and 25% water, while for **3**–**6** solutions were 73.2% methanol, 24.4% water and 2.4% DMF by volume. In all solutions $I = 0.10$ mol dm^{-3} ($NaClO_4$). [b] The K values determined by UV–visible spectrophotometry are <2 greater than those determined by fluorimetry. This may be because the concentrations of **2, 3** and **4** were 315, 10 and 10 times greater in the spectrophotometric studies than in the fluorimetric studies, respectively; while the $[Al^{3+}]_{total}$ ranges were similar in both types of study, the equilibrium shown in Scheme 2 and the analogous equilibria for **3** and **4** were slightly more to the left in the fluorimetric studies. [c] pK_as for the carboxylic acid and phenol groups, respectively. [d] Also 0.001 mol dm^{-3} in $HClO_4$.

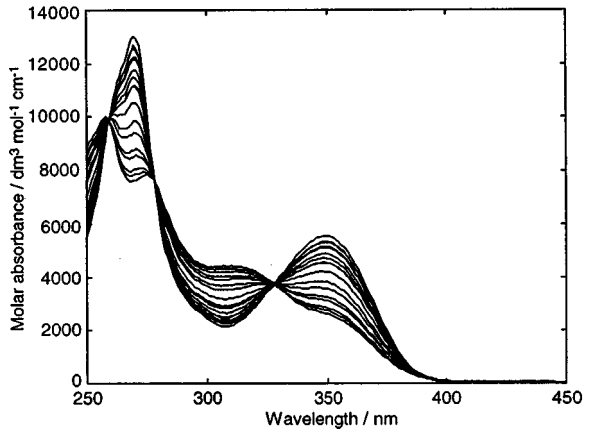


Fig. 1 Variation of the UV–visible absorption spectrum of 1.20×10^{-4} mol dm^{-3} **2** with added $Al(ClO_4)_3$ in 75% methanol and 25% water by volume solution, 0.10 mol dm^{-3} in $NaClO_4$ and 0.001 mol dm^{-3} in $HClO_4$ at 298.2 K. The absorbance increases at 270 and 350 and decreases at 300 nm as $[Al(ClO_4)_3]$ increases in the sequence: 0, 2.0×10^{-5}, 4.0×10^{-5}, 6.0×10^{-5}, 8.0×10^{-5}, 1.0×10^{-4}, 1.6×10^{-4}, 2.0×10^{-4}, 3.0×10^{-4}, 4.0×10^{-4}, 4.6×10^{-4}, 5.0×10^{-4}, 6.0×10^{-4}, 7.2×10^{-4}, 7.6×10^{-4}, 8.0×10^{-4}, 9.2×10^{-4}, 9.6×10^{-4}, 1.00×10^{-3} and 1.20×10^{3} mol dm^{-3}.

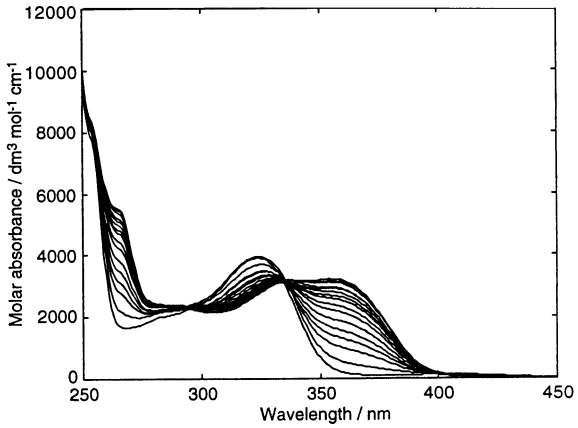


Fig. 3 Variation of the UV–visible absorption spectrum of 1.20×10^{-4} mol dm^{-3} **4** with added $Al(ClO_4)_3$ in 73.2% methanol, 24.4% water and 2.4% DMF by volume solution, 0.10 mol dm^{-3} in $NaClO_4$ at 298.2 K. The absorbance increases at 270 and 360 and decreases at 320 nm as $[Al(ClO_4)_3]$ increases in the sequence: 0, 2.0×10^{-5}, 6.0×10^{-5}, 1.0×10^{-4}, 1.5×10^{-4}, 2.0×10^{-4}, 3.0×10^{-4}, 4.0×10^{-4}, 5.0×10^{-4}, 6.0×10^{-4}, 7.0×10^{-4}, 8.0×10^{-4}, 9.0×10^{-4}, 1.00×10^{-3}, 1.20×10^{-3} and 1.40×10^{-3} mol dm^{-3}.

fluorescence data a formation constant $K = 1830 \pm 10$ dm^3 mol^{-1} is obtained. The fluorescence maxima for $[Al(\mathbf{2}')]^+$ occurs at 429 nm. The increased fluorescence of coordinated **2**′ probably reflects a combination of the increased rigidity introduced into its structure through coordination with the consequent loss of fluorescence quenching of vibrational and rotational modes, and fluorophore deprotonation and coordination, which increases aromatic character and the probability of n–π electronic transitions. A similar explanation probably applies for the increased fluorescence of coordinated **3**′ and **4**′ discussed below.

Ligands 3 and 4. The UV-visible spectra of both **3** and **4** vary systematically with $[Al^{3+}]_{total}$ as exemplified by **4** in Fig. 3. The spectrum of free **4** shows a shoulder at 254 (8300) and a maximum at 323 nm (3920 dm^3 mol^{-1} cm^{-1}). The spectrum of $[Al(\mathbf{4}')]^{2+}$ shows a shoulder at 270 (5490), a broad shoulder at 280–300 (2350) and a broad maximum at 335–360 nm (3200 dm^3 mol^{-1} cm^{-1}) when coordinated by Al^{3+}. The isosbestic points at 290 and 335 nm show the chromophore

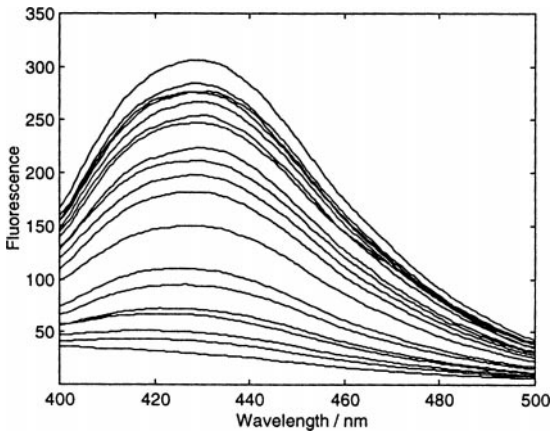


Fig. 2 Variation of the fluorescence of 3.80×10^{-7} mol dm^{-3} **2** with added $Al(ClO_4)_3$ in 75% methanol and 25% water by volume solution, 0.10 mol dm^{-3} in $NaClO_4$ and 0.001 mol dm^{-3} in $HClO_4$ at 298.2 K. The excitation wavelength was 328 nm. The fluorescence increases as $[Al(ClO_4)_3]$ increases in the sequence: 0, 2.0×10^{-5}, 4.0×10^{-5}, 6.0×10^{-5}, 8.0×10^{-5}, 1.0×10^{-4}, 1.6×10^{-4}, 2.0×10^{-4}, 3.0×10^{-4}, 4.0×10^{-4}, 4.6×10^{-4}, 5.0×10^{-4}, 6.0×10^{-4}, 7.2×10^{-4}, 7.6×10^{-4}, 8.0×10^{-4} 9.2×10^{-4}, 9.6×10^{-4}, 1.00×10^{-3} and 1.20×10^{-3} mol dm^{-3}.

experiences two dominant environments in the complexation equilibrium and $K = 7080 \pm 110$ dm^3 mol^{-1} for $[Al(\mathbf{4}')]^{2+}$ was obtained from absorbance data collected at 1 nm intervals over the range 340–380 nm. The spectrum of **3** shows a shoulder at 255 (11 000) and a maximum at 310 nm (4460 dm^3 mol^{-1} cm^{-1}). In $[Al(\mathbf{3}')]^{2+}$ the spectrum of **3**′ shows a shoulder at 270 (7300) and broad maxima at 320 (3570) and 345 nm (2970 dm^3 mol^{-1} cm^{-1}). Isosbestic points appear at 290 and 322 nm in the spectra of 1.20×10^{-4} mol dm^{-3} solutions of **3** containing increasing $[Al^{3+}]_{total}$ over the range 0–1.20×10^{-3} mol dm^{-3}, consistent with the chromophore experiencing two dominant environments in the complexation equilibrium, and $K = 5570 \pm 120$ dm^3 mol^{-1} was determined for $[Al(\mathbf{3}')]^{2+}$ from absorbance data collected at 1 nm intervals over the range 330–370 nm. Because the solutions of **3** and **4** studied were not buffered a small decrease in pH occurs as $[Al^{3+}]_{total}$ increases. This was not incorporated into either of the above stability constant determinations or those discussed below.

The isosbestic point at 335 nm was chosen as the excitation wavelength for the fluorescence study of the coordination of **4** by Al^{3+}. Free **4** has a very low fluorescence but coordination by Al^{3+} induces substantial fluorescence in **4**′ as seen in Fig. 4. The broad fluorescence band shows a systematic increase in intensity with increase in $[Al^{3+}]_{total}$, and these systematic changes in fluorescence collected at 1 nm intervals over the range 410–450 nm yielded $K = 3890 \pm 30$ dm^3 mol^{-1} for $[Al(\mathbf{4}')]^{2+}$. The fluorescence maximum for $[Al(\mathbf{4}')]^{2+}$ occurs at 430 nm. The isosbestic point at 322 nm was chosen as the excitation wavelength for the fluorescence study of the coordination of **3** by Al^{3+}. Free **3** also has a very low fluorescence but coordination of **3**′ by Al^{3+} induces substantial fluorescence. The fluorescence band of $[Al(\mathbf{3}')]^{2+}$ is broad with a maximum at 420 nm. The systematic increase in fluorescence intensity observed for 1.20×10^{-5} mol dm^{-3} solutions of **3** in which $[Al^{3+}]_{total}$ increased over the range 0–1.20×10^{-3} mol dm^{-3}, gave $K = 3190 \pm 40$ dm^3 mol^{-1} from fluorescence data collected at 1 nm intervals over the range 400–440 nm.

Ligands 5 and 6. The UV–visible spectra of both **5** and **6** exhibit significant changes with $[Al^{3+}]_{total}$ as exemplified by **5** in Fig. 5. The free ligand shows maxima at 280 (11 100) and 350 (4250), and **5**′ exhibits maxima at 280 (12 900) and 378 nm

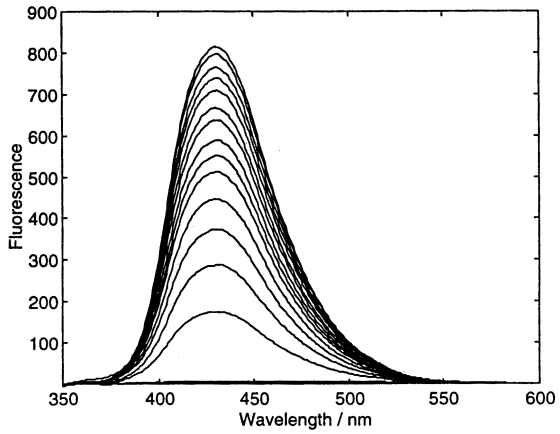


Fig. 4 Variation of the fluorescence of 1.20×10^{-5} mol dm^{-3} **4** with added $Al(ClO_4)_3$ in 73.2% methanol, 24.4% water and 2.4% DMF by volume solution, 0.10 mol dm^{-3} in $NaClO_4$ at 298.2 K. The excitation wavelength was 335 nm. The fluorescence increases as $[Al(ClO_4)_3]$ increases in the sequence: 0, 4.0×10^{-5}, 8.0×10^{-5}, 1.2×10^{-4}, 1.6×10^{-4}, 2.0×10^{-4}, 2.4×10^{-4}, 2.8×10^{-4}, 3.2×10^{-4}, 3.6×10^{-4}, 4.0×10^{-4}, 4.4×10^{-4}, 4.8×10^{-4}, 5.2×10^{-4} and 5.6×10^{-4} mol dm^{-3}.

(3940 dm^3 mol^{-1} cm^{-1}) when it is coordinated to Al^{3+}. The isosbestic points at 317 and 364 nm show the chromophore experiences two dominant environments in the complexation equilibrium and $K = 11\,960 \pm 430$ dm^3 mol^{-1} was derived for $[Al(\mathbf{5}')]^{2+}$ from absorbance data collected at 1 nm intervals over the range 370–410 nm. In the free state **6** shows a maximum at 318 (9400), which changes to 337 (13 300) and a shoulder appears at 375–400 nm (3500 dm^3 mol^{-1} cm^{-1}) when **6′** is coordinated by Al^{3+}. The isosbestic points at 265, 316 and 380 nm show the chromophore experiences two dominant environments in the complexation equilibrium and $K = 44\,800 \pm 2000$ dm^3 mol^{-1} for $[Al(\mathbf{6}')]^{2+}$ derived from absorbance data collected at 1 nm intervals over the range 320–360 nm for 1.20×10^{-4} mol dm^{-3} solutions of **6** in which $[Al^{3+}]_{total}$ increased over the range 0–1.20×10^{-3} mol dm^{-3}.

Neither **5** nor **6** are fluorescent in the free state and the Al^{3+} complexes of **5′** and **6′** show no fluorescence. This is attributable to the formation of twisted intramolecular charge transfer (TICT) states by these ligands.[19–21] The nitroaromatic units of **5** and **6** undergo intramolecular charge transfer in the excited state with the aromatic ring and nitro group acting as the electron donor and acceptor, respectively. In the ground state and the initial excited state the aromatic and nitro groups are coplanar and achieve maximum conjugation. However, a TICT excited state may form from the initial excited state where the planes of the aromatic ring and the nitro group are twisted at 90° to each other, conjugation between the two entities is lost and complete transfer of an electronic charge between them results. Sometimes emission may be observed from both the initial and TICT excited states. This is observed for neither **5** nor **6** in the free state and for neither **5′** nor **6′** in the coordinated state, consistent with the occurrence of non-emissive decay to the ground state through energy dissipation into vibrational modes.

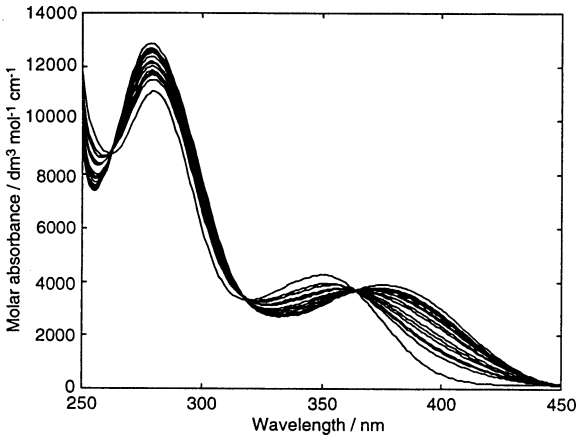


Fig. 5 Variation of the UV–visible absorption spectrum of 1.21×10^{-4} mol dm^{-3} **5** with added $Al(ClO_4)_3$ in 73.2% methanol, 24.4% water and 2.4% DMF by volume solution, 0.10 mol dm^{-3} in $NaClO_4$ at 298.2 K. The absorbance increase at 270 and 360 and decreases at 320 nm as $[Al(ClO_4)_3]$ increases in the sequence: 0, 3.0×10^{-5}, 6.0×10^{-5}, 9.0×10^{-5}, 1.2×10^{-4}, 1.6×10^{-4}, 2.0×10^{-4}, 3.0×10^{-4}, 4.0×10^{-4}, 5.0×10^{-4}, 6.0×10^{-4}, 7.0×10^{-4}, 8.0×10^{-4}, 9.0×10^{-4}, 1.00×10^{-3} and 1.20×10^{-3} mol dm^{-3}.

Coordination of ligands 2–6 by other metal ions

Under the same acidic conditions as for the study of Al^{3+} coordination of **2** described above, the spectrum of **2** showed very small increases in absorbance over the range 250–370 nm in the presence of 1.2×10^{-3} mol dm^{-3} Zn^{2+}, and moderate absorbance increases in the presence of 1.2×10^{-3} mol dm^{-3} Cd^{2+}. However, these changes were very small compared to those induced by Al^{3+}, consistent with a weaker coordination of these two ions. In the absence of added $HClO_4$, but otherwise under the same conditions, Zn^{2+} induced slightly larger changes in the spectrum of **2**, and the spectral changes induced by Cd^{2+} also increased, but still Al^{3+} induced much greater changes in the spectrum of **2**. (The pH of the latter solutions were 4.3, 5.4 and 5.0 for Al^{3+}, Zn^{2+} and Cd^{2+}, respectively, consistent with decreasing protolysis of coordinated water as the surface charge density of the metal ion decreases.) Under neither set of conditions did the spectrum of **2** show significant change in the presence of the biologically important K^+, Mg^{2+} and Ca^{2+} ions. Overall, these data are qualitatively consistent with **2** in its dianionic form, **2′**, showing significant selectivity for complexing Al^{3+}.

Under the same conditions as for the Al^{3+} studies, the spectra of **3–6** showed very small increases in absorbance over the range 250–370 nm in the presence of 1.2×10^{-3} mol dm^{-3} Zn^{2+}, and slightly larger absorbance increases in the presence of 1.2×10^{-3} mol dm^{-3} Cd^{2+} and Pb^{2+}. Compared with the changes induced by Al^{3+}, these changes were very small, consistent with a stronger coordination of Al^{3+}. Neither K^+, Mg^{2+} nor Ca^{2+} induced significant spectral changes. These data are qualitatively consistent with **3–6** showing significant selectivity for complexing Al^{3+}.

For a ligand to be useful as a fluorophore in the detection of a given metal ion it has to possess substantial selective complexing character for that metal ion. Given the close relationship of **2–6** to pyochelin, **1**, it is probable that these ligands will strongly complex Fe^{3+}. However, because the d–d electronic transitions of this d^5 ion quench ligand fluorescence the coordination of **2–6** by Fe^{3+} was not studied. For the same reason other transition metal ions with d^1–d^9 electronic configurations were not examined. However, the data reported here indicates that biologically ubiquitous Zn^{2+} could interfere with the detection of intracellular Al^{3+} and accordingly we propose to modify our fluorophores to enhance their selectivity for Al^{3+} for such use.

Experimental

Potentiometric titrations were carried out using an Orion Ross Sureflow 81-72 BN combination electrode, an Orion SA 720 potentiometer and a Metrohm E665 Dosimat autoburette

interfaced to an XT/4-8086 computer. The reference compartment of the combination electrode was filled with 0.10 mol dm^{-3} $NaClO_4$ in 75% methanol and 25% water (v : v) solvent and allowed to attain equilibrium over 2 days before use. The pK_as of **2** and **3**–**6** were determined from triplicate titrations of 10 cm^3 of 1.00×10^{-3} mol dm^{-3} ligand in 75% methanol and 25% water by volume and 73.2% methanol, 24.4% water and 2.4% DMF by volume solutions, respectively, thermostatted at 298.2 K in a titration vessel against 0.10 mol dm^{-3} NaOH in 75% methanol and 25% water by volume solution delivered from a microburette. A fine stream of nitrogen, which had been bubbled through successive traps containing 0.10 mol dm^{-3} sodium hydroxide and 75% methanol and 25% water by volume solvent, respectively, was bubbled continuously through the magnetically stirred titration solution to remove carbon dioxide. Ligand pK_as were derived from the titration data using the programme SUPERQUAD[22] on a Digital Venturis 575 computer.

UV–visible spectra were measured from 250 to 450 nm at 1 nm intervals at a scan rate of 1 nm s^{-1} and a spectral bandwidth of 1.0 nm with a Varian Cary 2200 spectrophotometer interfaced to an IBM-compatible 486DX personal computer. Fluorescence spectra were measured from 350 to 600 nm at 0.5 nm intervals at a scan rate of 4 nm s^{-1} with a Perkin Elmer LS50B fluorimeter interfaced to a Digital 75 MHz Pentium computer. The entrance and exit slit widths were adjusted in the range 0.4 to 0.6 nm for each ligand according to the intensity of emitted light. Solutions were thermostatted at 298.2 K in 1 × 1 cm quartz cells for both sets of measurements. Stability constants were determined by fitting algorithms for 1 : 1, 1 : 1 and 1 : 2 and 1 : 1 and 2 : 1 complexations (Al^{3+} : ligand) to the absorbance and fluorescence data using an inhouse programme, which also generated the coordinated ligand spectra. The 1 : 1 algorithm yielded the better data fits for each system.

Uncorrected melting points were determined using a Kofler hot-stage apparatus attached to a Reichert microscope. Infrared (IR) spectra were recorded in nujol mulls on either a Hitachi 270-30 grating spectrophotometer or an ATI Mattson Genesis FT spectrophotometer. Thin layer chromatography (TLC) was carried out on Kieselgel 60 F254 (Merck) on aluminium backed plates. NMR spectra were recorded on a Varian Gemini 200 spectrophotometer operating at 199.8 MHz (^1H) and 50.4 MHz (^{13}C). Mass spectra were recorded using a VG ZAB 2HF spectrometer operating at 70 eV.

Syntheses

N,*N*-Dimethylformamide (Aldrich) was distilled under reduced pressure and stored under dry nitrogen. Water was purified using the Milli-Q reagent system. Bulk methanol was purified by distillation prior to use. The reagents used in ligand preparations were either of good commercial quality or were prepared by standard methods. Ligands **2** and **3** were prepared by literature methods and were characterised by IR spectroscopy, NMR spectroscopy and molecular mass.[17,18]

4-Bromo-2-(4,5-dihydro-1,3-thiazol-2-yl)phenol, 4. A solution of 2-aminoethanethiol hydrochloride (397 mg, 3.5 mmol), triethylamine (1.1 cm^3, 7.89 mmol) and 5-bromo-2-hydroxybenzonitrile (610 mg, 3.08 mmol) in ethanol (8 cm^3) was heated at reflux for 2.5 h. The solution was cooled to room temperature and water (15 cm^3) was added. The yellow precipitate was collected by vacuum filtration and dried for 24 h over P_2O_5 to yield **4**, (0.609 g, 76%). MP 113–118 °C. (Found: C, 41.97; H, 3.08; N, 5.43; S, 12.58%. C_9H_8NOSBr requires C, 41.88; H, 3.12; N, 5.43; S, 12.42%) IR: 2674 (OH), 1592 (C=C), 1016 cm^{-1} (CN). MS *m*/*z* 259/257 (M^+, 100/98.5%). ^1H NMR ($CDCl_3$) δ: 12.72, s, OH; 7.52, d, *J* 2.4 Hz, H3; 7.42, dd, *J* 2.4, 8.8 Hz, H5; 6.90, d, *J* 8.8 Hz, H6; 4.49, t, *J* 8.4 Hz, NCH_2; 3.40, t, *J* 8.4 Hz, SCH_2. ^{13}C NMR ($CDCl_3$) δ: 171.55, C=N; 158.32, C1; 135.55, 132.84, C5, C3; 119.03, C6; 117.90, 110.19, C2, C4; 63.36, CH_2N; 32.06, CH_2S.

2-(4,5-Dihydro-1,3-thiazol-2-yl)-5-nitrophenol, 5. A solution of 2-hydroxy-4-nitrobenzonitrile (2.26 g, 0.013 mol), 2-aminoethanethiol hydrochloride (1.90 g, 0.016 mol) and triethylamine (2.71 g, 0.026 mol) in ethanol (50 cm^3) was degassed and placed under a nitrogen atmosphere. The solution was refluxed for 50 min and cooled to room temperature, forming crystals of **5**, which were collected by vacuum filtration and washed with ethanol (1.95 g, 63%). MP 150–151 °C. (Found: C, 48.22; H, 3.37; N, 12.52; S, 14.45%. $C_9H_8N_2O_3S$ requires C, 48.21; H, 3.60; N, 12.49; S, 14.30%) IR: 2724 (OH), 1614 (C=C), 1573 (C=C), 1340 cm^{-1} (CO). MS *m*/*z*: 224 (M^+). ^1H NMR ($CDCl_3$) δ: 13.13, s, OH; 7.83, d, *J* 2.2 Hz, H6; 7.71, dd, *J* 2.2, 8.6 Hz, H4; 7.55, d, *J* 8.6 Hz, H3; 4.59, t, *J* 8.6 Hz, NCH_2; 3.56, t, *J* 8.4 Hz, SCH_2. ^{13}C NMR ($CDCl_3$) δ: 171.86, C=N; 159.87, C1; 150.24, C4; 131.48, C3; 121.01, C2; 113.26, 112.52, C6, C4; 63.46, NCH_2; 32.11, SCH_2.

2-(4,5-Dihydro-1,3-thiazol-2-yl)-4-nitrophenol, 6. A solution of 2-hydroxy-5-nitrobenzonitrile (987 mg, 6.02 mmol), 2-aminoethanethiol hydrochloride (845 mg, 7.44 mmol) and triethylamine (1.2 g, 11.77 mmol) in ethanol (25 cm^3) was degassed and placed under a nitrogen atmosphere. The solution was refluxed for 2 h after which a precipitate began to form. The reaction mixture was cooled to room temperature and yellow needle crystals of **6** were collected by vacuum filtration and washed with ethanol (820 mg, 61%). MP 163–166 °C. (Found: C, 48.06; H, 3.36; N, 12.52; S, 14.48%. $C_9H_8N_2O_3S$ requires C, 48.21; H, 3.60; N, 12.49; S, 14.30%) IR: 3000 (OH), 1594 (C=C), 1334 cm^{-1} (CO). MS *m*/*z*: 224 (M^+). ^1H NMR ($CDCl_3$) δ: 13.80, s, OH; 8.33, d, *J* 2.8 Hz, H3; 8.24, dd, *J* 3.0, 9.0 Hz, H5; 7.07, d, *J* 9.4 Hz, H6; 4.53, t, *J* 8.2 Hz, NCH_2; 3.48, t, *J* 8.2 Hz, SCH_2. ^{13}C NMR ($CDCl_3$) δ: 172.20, CN; 164.76, C1; 139.64, C4; 128.19, 126.98, C5, C3; 117.98, C6; 115.78, C2; 63.17, NCH_2; 32.24, SCH_2.

Acknowledgements

Funding by the Australian Research Council is gratefully acknowledged.

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