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Selective signalling of molybdate by a siderophore derivative†

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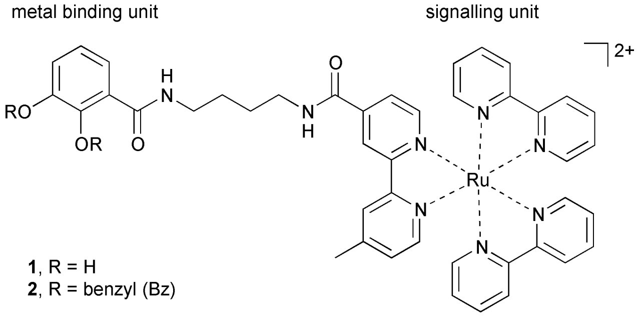
By connecting the siderophore aminochelin of *Azotobacter vinelandii* to a highly fluorescent tris(2,2′-bipyridyl)-ruthenium(II)-type chromophore, a new modular sensor reagent has been synthesised and characterised, which selectively signals the presence of molybdate in solution through luminescence quenching.

The design of luminescent molecular systems for the detection of anionic substrates of biological and environmental concern is a rapidly developing area. A variety of anions have been targeted, including NO_3^-, $H_2PO_4^-$, HPO_4^{2-} and ATP^{n-}.[1,2] However, studies addressing *metal* containing oxoanions are still rare and receptors used so far, for example for TcO_4^- and ReO_4^-, rely on electrostatic interactions and hydrogen bonding.[3]

Luminescent signalling offers the advantage of high sensitivity and applicability in fibre-optic probes. Modular sensor reagents that consist of an ion-responsive receptor and a highly emissive chromophore separated by a spacer allow a rational sensor design, based on fast signalling processes, such as photoinduced intramolecular electron or energy transfer.[4] We followed this modular approach in the design of the new sensor system **1** for the oxoanion molybdate (MoO_4^{2-}), the predominant form of molybdenum under oxidising conditions and in dilute aqueous solutions at neutral pH. Since molybdenum plays a fundamental role in chemical and biological processes, a sensitive and selective chemosensor for molybdate could have applications in industrial control, environmental monitoring and biochemistry.[5]



1, R = H
2, R = benzyl (Bz)

The metal binding unit of **1** consists of the naturally occurring siderophore aminochelin [*N*-(2,3-dihydroxybenzoyl)-diaminobutane] of *Azotobacter vinelandii*. Recently, luminescent hexadentate siderophore analogues have been used successfully for quantitative determinations of Fe(III) in aqueous solutions.[6] However, siderophores of lower denticity bind not only Fe(III) but also Mo(VI)[7] with high affinity. Since the reaction of the oxo**anion** MoO_4^{2-} with catechols shows a pH-dependency different from the reactions of **cations** such as Fe^{3+} with catechols, pH-control offers a way of selecting molybdate (Scheme 1). In addition, the positively charged $[Ru(bipy)_3]^{2+}$ (bipy = 2,2′-bipyridine) signalling unit supports **anion** binding by electrostatic attraction[2] and thereby enhances the sensor's affinity for molybdate. The $[Ru(bipy)_3]^{2+}$-chromophore has been chosen not only for its luminescence but also because of the photoinduced electron transfer (PeT) processes observed in combination with appended catechols.[8–10]

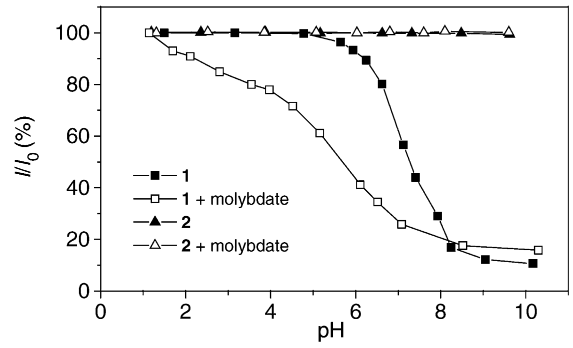
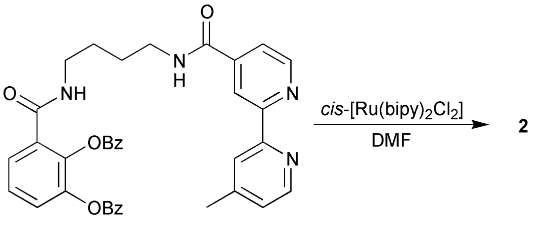


Fig. 1 pH dependence of the relative intensity of 2×10^{-7} M reagent solutions containing: **1** (■); **1** + 0.5 equiv. MoO_4^{2-} (□); **2** (▲); **2** + 0.5 equiv. MoO_4^{2-} (△). $\lambda_{exc} = 460$ nm, $\lambda_{em} = 615$ nm.§

$$MoO_4^{2-} + 2\,catH_2 \rightleftharpoons [MoO_2(cat)_2]^{2-} + 2\,H_2O$$
$$Fe^{3+} + 3\,catH_2 \rightleftharpoons [Fe(cat)_3]^{3-} + 6\,H^+$$

Scheme 1

The synthesis of **1** involved HBTU-mediated coupling of benzyl-protected aminochelin[11] with 4′-methyl-2,2′-bipyridyl-4-carboxylic acid.[12] The resulting hybrid ligand **3** (Scheme 2) was refluxed with *cis*-$[Ru(bipy)_2Cl_2]$ in DMF to obtain the benzyl-protected ruthenium complex **2** which was subsequently deprotected by catalytic hydrogenation (5% Pd/C) to give the target compound **1** in 70% overall yield.‡



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Scheme 2

The emission behaviour of **1** and **2** in aqueous acetonitrile solutions was investigated in the pH-range 2–11 by titrations with standard base solution.§ The fluorescence intensity *vs.* pH-profiles obtained are shown in Fig. 1. While the metal-to-ligand charge-transfer emission intensity of the benzyl-protected derivative **2** is pH-independent, the free sensor **1** shows a sigmoidal decrease in luminescence intensity corresponding to a pK_a of *ca.* 7.1, which is in agreement with the pK_a of 7.34 reported for *N*-ethyl-2,3-dihydroxybenzamide.[13] Electron rich phenolates have reducing properties and can

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† Electronic supplementary information (ESI) available: synthesis and characterisation data for **1**–**3**; emission spectrum for **1** in the presence of varying amounts of Fe(III) and molybdate. See http://www.rsc.org/suppdata/dt/b1/b104970b/

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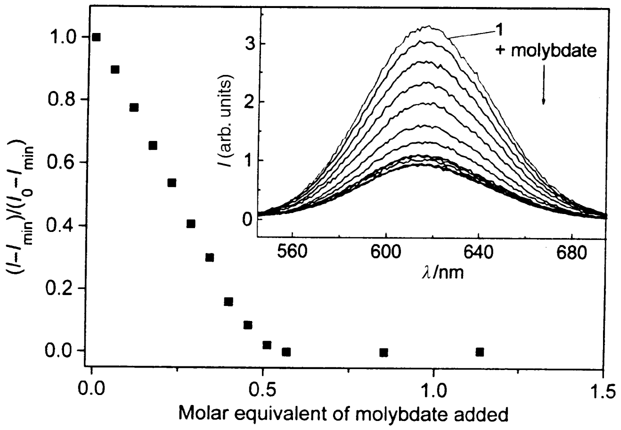

Fig. 2 Variation of the fluorescence intensity ratio at 615 nm as a function of molar molybdate fractions at pH 5.7 (average of two independent titrations). The inset shows the corresponding emission spectra. Buffer 2,6-lutidine, $\lambda_{exc} = 460$ nm.

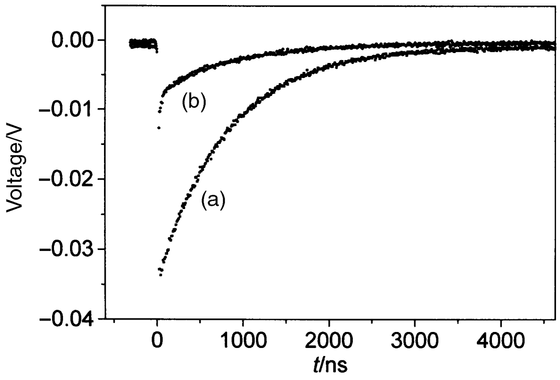

Fig. 3 Time-resolved emission decay profiles for a) **1** and b) **1** + Na_2MoO_4 (1 : 1), $\lambda_{exc} = 355$ nm, $\lambda_{em} = 615$ nm.

transfer an electron to a proximate photogenerated Ru^{III}-(bipy$^{•-}$) moiety, leading to luminescence quenching.[10]

Analogous pH-dependent experiments were carried out with solutions also containing 0.5 equiv. of molybdate. As is evident from Fig. 1, the emission intensity of **1** decreases at significantly lower pH in the presence of MoO_4^{2-} than in its absence. This quenching effect can be related to the co-ordination of molybdenum to the catecholate unit of **1**. The end point of the titration of **1** with molybdate in buffered solution at pH 6.7 is consistent with a molybdenum : catecholate 1 : 2 complex (Fig. 2). The fact that the emission intensity of the non-complexing derivative **2** is not affected by the presence of molybdate (Fig. 1) further confirms that the quenching observed in the case of **1** is caused by molybdenum binding and is not due to a diffusion controlled, intermolecular process.

Fluorescence measurements were also performed at low-temperature. Aqueous acetonitrile solutions containing a) **1** and b) **1** and 0.5 equiv. of molybdate were adjusted to pH 9 and pH 7, respectively, to ensure that substantial quenching occurs. Cooling the solutions to 77 K gave glasses and largely restored the emission of the signalling unit in both cases. Such luminescence revival indicates that the quenching is due to electron transfer (eT) and not energy transfer (ET) processes.[14]

A preliminary time-resolved emission study of **1** and its molybdenum complex was conducted using 355 nm Nd : YAG excitation (10 ns pulsewidth, Fig. 3). The decay of the MLCT excited state of **1** was fitted adequately by a single-exponential function, giving a lifetime of 710 ± 30 ns, which is in accordance with the lifetimes typically observed for $[Ru(bipy)_3]^{2+}$ complexes. In the presence of molybdate, the decay profiles were found to fit to dual-exponential functions. The longer lifetime corresponds to free **1**, while the shorter lifetime (*ca.* 30 ns) corresponds to the molybdenum complex of **1**. The proportion of the two functions altered with molybdate concentration.

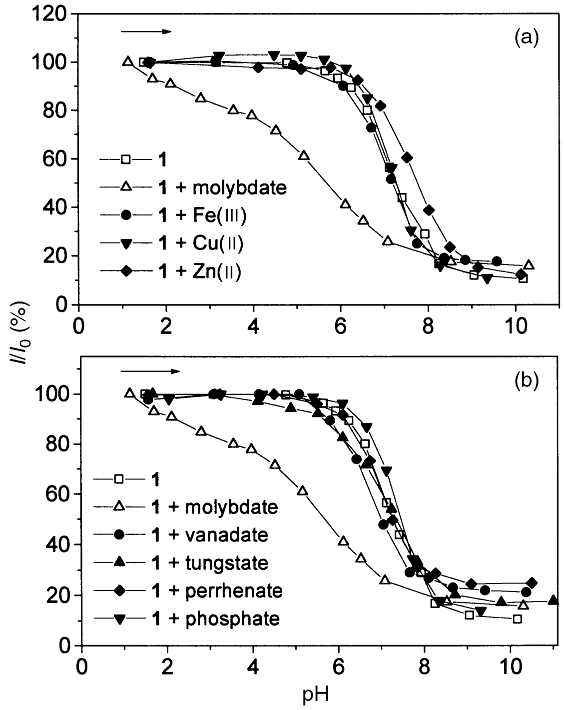

Fig. 4 pH dependence of the relative emission intensity of solutions containing: a) **1**, **1** + molybdate (0.5 equiv.), Fe^{3+} (0.3 equiv.), Cu^{2+} (0.3 equiv.) or Zn^{2+} (0.5 equiv.); b) **1** and **1** + the indicated anions (0.5 equiv. each). $\lambda_{exc} = 460$ nm, $\lambda_{em} = 615$ nm.

In a further step, the selectivity of **1** was investigated by titrating the acidic sensor solution with standard base in the presence of potentially interfering cations or anions. The emission intensity *vs.* pH-profiles obtained in the presence of metal cations, such as Fe^{3+}, Cu^{2+} or Zn^{2+} superimpose well on that observed for the titration of **1** alone (Fig. 4a). The lack of interactions may be rationalised by the electrostatic repulsion between the metal cations and the positively charged sensor molecule. Remarkably, even the addition of oxoanions, such as PO_4^{3-}, ReO_4^{-}, VO_4^{3-} and WO_4^{2-} did not alter the pH-profile significantly (Fig. 4b). This demonstrates that selective binding and signalling of molybdate can also be achieved by taking advantage of the characteristic co-ordination chemistry of molybdenum.

According to the pH-profiles obtained, the highest selectivity for molybdate can be achieved between pH 4.8 and pH 5.8. In fact, by using a buffered solution of **1** at pH 5.7, no significant luminescence quenching was observed on addition of Fe(III), see supplementary information (ESI †) (or vanadate), while the subsequent addition of molybdate to the same solution resulted in a significant intensity decrease (like that shown in Fig. 2).

In summary, the biomimetic modular system **1** represents a promising prototype of a new class of luminescent sensor reagents for molybdate. Under pH control, **1** selectively binds MoO_4^{2-} and signals its presence and concentration by a decrease in emission intensity. The selectivity is based on a combination of metal–ligand bonding and electrostatic interactions. Interesting applications should be possible by modification of the prototype, *e.g.* through an improvement of its water solubility or its immobilisation at the tip of a fibre-optic probe. Studies directed towards the determination of stability constants and the improvement of the water compatibility of the system are underway.

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Notes and references

‡ ^{1}H-NMR (500 MHz, 313 K, DMSO-d$_6$) δ 1.55 (s, broad, 4H, $CH_2(CH_2)_2CH_2$), 2.48 (s, 3H, CH_3), 3.21 (m, broad, 2H, CH_2CH_2NH),

3.31 (m, broad, 2H, CH_2CH_2NH), 6.59 (t, 1H, cat-H^5), 6.83 (d, 1H, cat-H^4), 7.29 (d, 1H, cat-H^6), 7.34 (d, 1H, bpy-H), 7.43–7.48 (m, 5H, bpy-H), 7.55 (d, 1H, bpy-H), 7.65 (d, 4H bpy-H), 7.72 (t, 1H, bpy-H), 7.79 (d, 1H, bpy-H), 8.08–8.12 (m, 4H, bpy-H), 8.70 (s, broad, 1H, bpy-H), 8.75 (d, 4H, bpy-H), 8.94 (t, broad, 1H, NH), 9.4 (s, broad, 1H, NH). Further characterisation data and synthetic details are available as supplementary information (ESI †)

§ The titrations were conducted in air-equilibrated MeCN–H_2O (15 : 1) solutions at 20 °C. Titrations in pure H_2O could not be carried out due to the poor solubility of **1**. The pH-scale was calibrated by using Gran's method.[15] For the titration of **1** in the presence of molybdate a non-sigmoidal shape of the intensity *vs.* pH profile is observed. This may be attributed to pH-dependent equilibria involving molybdenum complexes of different stoichiometry at lower pH.

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