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Switch-on luminescence detection of steroids by tris(bipyridyl)ruthenium(II) complexes containing multiple cyclodextrin binding sites†

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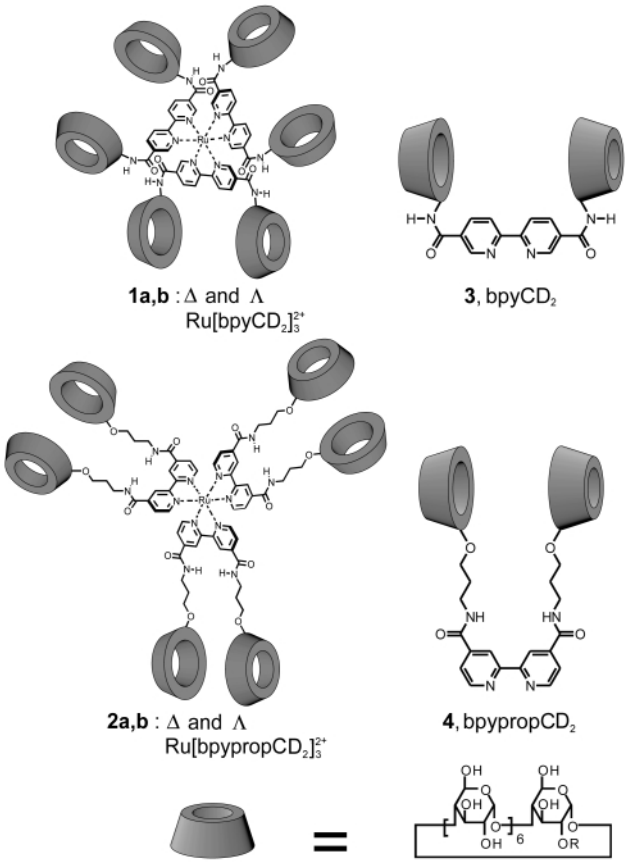
A luminescent ruthenium(II) complex with six cyclodextrin binding sites is shown to switch off its emission upon binding of *N,N′*-dinonyl-4,4′-bipyridinium bromide and to recover luminescence upon displacement of the bipyridinium ion by a steroid.

The design of luminescent cyclodextrin sensor systems which are able to detect neutral organic compounds, *e.g.* steroids by a change in emission properties is an area which has attracted much interest over the last decade.[1] Most of these systems rely on a decrease in fluorescence intensity when a covalently linked fluorophore is displaced from the cyclodextrin cavity by a guest. Few systems exist in which the detection of a guest is accompanied by an increase in emission intensity.[2,3] Here, we describe the sensor properties of two novel cyclodextrin-based sensor compounds, containing a central luminescent tris(bipyridyl) ruthenium(II) complex,[4] to which six cyclodextrins are attached. One of these compounds (**2**) is able to detect steroids by first turning off the emission of the ruthenium complex by the binding of a quenching viologen in its cyclodextrin cavities, and then switching on the original luminescence by displacement of the viologen by the steroid.

The orange ruthenium complexes **1** and **2** were synthesised by reaction of $RuCl_3$ with 3 equivalents of the dimers **3**[5] and **4**,‡ respectively, in boiling water. The resulting mixtures were poured into acetone to precipitate the complexes as their chloride salts.‡ The UV–VIS spectra of **1** and **2** showed a characteristic metal-to-ligand charge transfer (MLCT) absorption centered at 465 nm and an intense ligand centered (LC) absorption around 300 nm. No quenching of the emission by oxygen was observed for either compound probably because the ruthenium centers are shielded by the cyclodextrins.[6]

Compound **1** was only weakly luminescent (95% less intense than **2**), probably because the bulky cyclodextrins prevent optimal coordination of the bipyridyl ligands to the ruthenium centers. Compound **2** displayed a bright luminescence, twice as intense as the reference compound $Ru(bpy)_3^{2+}$ (bpy = 2,2′-bipyridine). Compound **2** was, therefore, selected for binding studies with organic guest molecules. When ursodeoxycholic acid, lithocholic acid, cholesterol or 1-adamantanecarboxylic acid were added to a solution of **2** in water no change in the emission spectrum of **2** was observed. This means that there is no direct or indirect communication between the ruthenium complex and these guest molecules when they are bound. Since it is known[7] that covalently linked viologens (*N,N′*-dialkyl-4,4′-bipyridiniums) are able to quench the emission intensity of tris(bipyridyl)ruthenium(II) complexes very efficiently *via* an electron transfer process, we chose *N,N′*-dinonyl-4,4′-bipyridinium bromide as a guest molecule. The two long alkyl chains were attached to this molecule to increase its binding to the cyclodextrins.[8] Upon addition of the bipyridinium compound to an aqueous solution of **2** the emission intensity gradually dropped, resulting in 92% quenching at saturation (Fig. 1). Most importantly, when this guest was added to the reference compound $Ru(bpy)_3^{2+}$ under the same conditions (aereated solution, same concentrations) only a linear decrease of 15% in luminescence was observed. This implies that the bipyridinium compound is bound in the cyclodextrin cavities and thereby facilitates electron transfer.



Analysis of the complex stoichiometry by constructing a Job-plot (see ESI†) suggested that a 1:1 complex was formed between **2** and the bipyridinium guest. The observed binding curve could be fitted to a 1:1 binding model and gave an apparent association constant (K_a) of 2.8×10^5 M^{-1}.

A microcalorimetric titration study of the binding of the bipyridinium guest in **2** revealed a different picture (Fig. 2). This time the observed titration curve could not be fitted to a 1:1 model, instead a second (**2**:bipyridinium = 1:2) equilibrium was needed to give a satisfactory fit (Fig. 2, inset). The data from this study are collected in Table 1. As can be seen the binding constant for the 1:1 complex matches within experimental error (15%) the observed value from the fluorimetric determination. Apparently, with the latter method, only the

† Electronic supplementary information (ESI) available: characterisation of compounds **2** and **4**, and a Job-plot for the binding of the *N,N′*-dinonyl-4,4′-bipyridinium bromide to **2**. See http://www.rsc.org/suppdata/cc/b0/b000271m/

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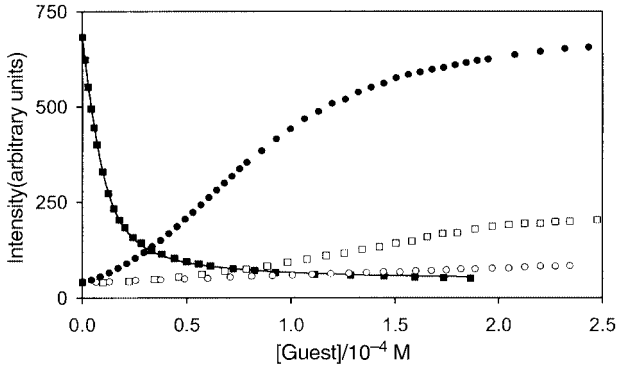

Fig. 1 Fluorimetric titration of (■) *N*,*N*′-dinonyl-4,4′-bipyridinium to **2** (1.0×10^{-5} M). The solid line shows the fitted curve assuming a 1:1 host(**2**):guest complex. Similar titration plots of (●) ursodeoxycholic acid, (□) lithocholic acid and (○) cholesterol to **2** (1.0×10^{-5} M) in the presence of 5.7×10^{-4} M of *N*,*N*′-dinonyl-4,4′-bipyridinium bromide. All titrations were performed in an aqueous 0.1 M Tris–HCl buffer at pH 7.0.

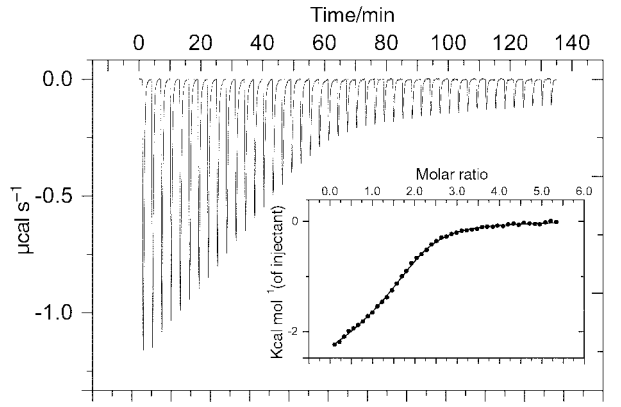

Fig. 2 Microcalorimetric titration of *N*,*N*′-dinonyl-4,4′-bipyridinium to **2** (1.0×10^{-4} M). For conditions see Table 1. The solid line (inset) shows the fitted curve according to a 1:2 (**2**:bipyridinium) model.

formation of the 1:1 complex can be detected. This implies that the binding of the first bipyridinium center results in almost complete quenching of the luminescence of **2** and that the second bipyridium unit has no effect on the luminescence.

Table 1 shows that $-T\Delta S$ of binding is the same for the 1:1 and the 1:2 complex. This parameter is probably related to the break up of solvent shells around the alkyl tails of the bipyridinium guest, which will be the same for the first and the second bipyridinium unit. By contrast ΔH of binding is different, being 1 kcal mol^{-1} smaller for the second step. We propose that two cyclodextrin moieties are involved in the complexation of a long chain bipyridinium guest.[8] Apparently, the binding of a second guest in **2** is disfavoured and the binding of a third guest completely absent, probably owing to a negative allosteric effect. Further studies are underway to substantiate this.

Having observed that bipyridinium compounds can quench the emission of **2** efficiently, we used this property to detect organic compounds. Displacement of the bipyridinium compound by other guests such as steroids should inhibit the quenching process and recover the luminescence of **2**. This would then give a sensor system which emits light when a guest is added. From a technological point of view this is an advantage since, in contrast to conventional systems, a luminescent signal is produced against a dark background, allowing a more sensitive detection in for instance a diagnostic test. When ursodeoxycholic acid was added to an aqueous solution of **2** and the bipyridinium ion, full recovery of the luminescence was observed (Fig. 1). A similar titration curve was recorded for lithocholic acid and cholesterol, showing the general applicability of the procedure. The curves for lithocholic acid and ursodeoxycholic acid displayed a sigmoidal shape suggesting that these steroids first displace one bipyridinium which will not result in a significant increase of the luminescence (*vide supra*), and only when the second bipyridinium ion becomes displaced the luminescence will be recovered.§

All steroids must have a binding constant of the same magnitude as the bipyridinium salt, *i.e.* 10^5 M^{-1} or slightly higher, in order for displacement in **2** to occur. The binding of ursodeoxycholic acid is strong enough to completely displace the bipyridinium salt and recover the full luminescence. Lithocholic acid only restores 30% of the emission, probably due to a weaker binding. The binding of cholesterol is too weak to even show a sigmoidal binding curve. Nevertheless, at a concentration of 2.3×10^{-4} M of cholesterol the luminescence has already doubled. Lithocholic acid shows a 5-fold increase at the same concentration, while for ursodeoxycholic acid the increase is 15-fold. This means that this sensor system is able to differentiate between different steroids which is a prerequisite for a selective diagnostic test. More detailed studies on this new sensor system are underway.

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Table 1 Thermodynamic data for the complexation of *N*,*N*′-dinonyl-4,4′ bipyridinium bromide in **2**[a]

Host–guest complex	K_a/M^{-1}	ΔH/kcal mol^{-1}	$-T\Delta S$/kcal mol^{-1}
1:1	2.4×10^5	−2.4	−4.8
1:2	4.0×10^5	−1.4	−4.9

[a] Obtained from a microcalorimetric titration of the bipyridinium guest with **2**. The data points were fitted assuming a two step (**2**:guest = 1:1 and 1:2) equilibrium. The titration was performed at 25.0 °C in an aqueous 0.1 M Tris–HCl buffer of pH 7.0, by adding small aliquots of a solution of the bipyridinium salt (3.09×10^{-3} M) to a solution of **2** (1.00×10^{-4} M). The data were corrected for the dilution heat of the bipyridinium ion.

Notes and References

‡ Compounds **1**, **2** and **4** were fully characterised by ^{1}H NMR, MS and elemental analysis.

§ In addition to compound **2** we also prepared a ruthenium complex with two unsubstituted bipyridines and one molecule of **4** as ligands. This mixed complex has only one binding site for the bipyridinium guest and therefore a single competition event should take place. The emission properties of this complex turned out to be much weaker than those of **2**, and, therefore, were not further investigated.

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