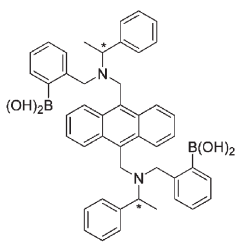
Enhanced fluorescence and chiral discrimination for tartaric acid in a dual fluorophore boronic acid receptor†

Jianzhang Zhao and Tony D. James*

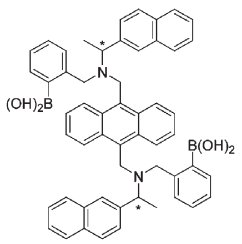
Received (in Cambridge, UK) 7th December 2004, Accepted 20th January 2005
First published as an Advance Article on the web 9th February 2005
DOI: 10.1039/b418279k

The addition of D-tartaric acid to *R,R*-2 causes a large increase in fluorescence. While addition of L-tartaric acid to *R,R*-2 only produces small changes in fluorescence.

Much recent attention has been paid to the development of synthetic molecular receptors with the ability to recognise, selectively, small molecules involved in biological pathways. Fluorescent sensors are preferred because they are well suited to meet the need for *in vivo* probes, such as mapping the spatial and temporal distribution of the biological analytes.[1,2] In particular boronic acid receptors have attracted considerable interest due to their ability to bind guests in aqueous media.[3–6] Fluorescent boronic acid based sensors for tartaric acid,[7,8] D-glucuronic acid[9,10] and D-glucaric acid[11] have been reported, as has a boronic acid based colorimetric indicator-displacement assay for the determination of the enantiomeric excess of α-hydroxy acids.[12] Hydrogen bonding receptors for the binding of tartaric acid,[13] chiral discrimination of hydroxylcarboxylates[14] and tartaric acid[15,16] are also known.



(*R*, *R*)-(-)-**1** and (*S*, *S*)-(+)-**1**



(*R*, *R*)-(-)-**2** and (*S*, *S*)-(+)-**2**

We have recently prepared enantioselective fluorescent sensors for sugar acids based on BINOL. The rigid axial chirality of BINOL was key in the selective chiral sensor's construction (functioning as chirogenic center and fluorophore).[17] Unfortunately, in this case, the chiral center is not in close proximity to the receptor's binding site, and BINOL has poor fluorescence properties. Therefore, we designed fluorescent chiral sensors *R,R*-**1** and *S,S*-**1**, which have two chiral centers in close proximity to the binding site of the receptor and used anthracene, a good fluorophore, as a rigid linker. This system displayed good chiral discrimination for a number of sugar acids including, tartaric acid.

Previously, we have used fluorescence resonance energy transfer (FRET) to improve the fluorescence response of a saccharide sensor towards D-glucose.[18] With this work we decided to explore the possibility of using FRET to enhance the fluorescence response towards one chiral form of tartaric acid.

Herein, we report the tight and selective binding of D- and L-tartaric acid with chiral fluorescent sensors *R,R*-**2** and *S,S*-**2**. The pH titration curves of *R,R*-**2** ($pK_a = 5.87 \pm 0.08$), *R,R*-**2** with L-tartaric acid ($pK_a = 8.56 \pm 0.05$) and *R,R*-**2** with D-tartaric acid ($pK_a = 7.46 \pm 0.03$) are shown in Fig. 1. From these curves it is clear that interaction of *R,R*-**2** with D-tartaric acid causes the largest fluorescence response. With *S,S*-**2** a mirrored response is observed and the largest fluorescence response is observed with L-tartaric acid.

From our previous work we know that *R,R*-**1** forms a stable cyclic complex with D-tartaric acid, while *S,S*-**1** forms a stable cyclic complex with L-tartaric acid.[19] The observed titration curves of *R,R*-**2** (blank) and *R,R*-**2** (L-tartaric acid) display reduced (quenched) fluorescence response due to close contact of the naphthalene and anthracene fluorophores. While the complex between *R,R*-**2** and D-tartaric acid results in a rigid structure where the fluorophores are unable to make close contact, and hence a larger fluorescence response is observed.

In order to probe the effect of the geometry of the receptor complexes on the fluorescent properties of the system we performed titrations of *R,R*-**1**, *S,S*-**1**, *R,R*-**2** and *S,S*-**2** with D- tartaric acid at pH 2.5, 5.6 and 7.0 (Table 1). At pH 2.5 the amine nitrogen of sensors **1** and **2** is fully protonated, thus,

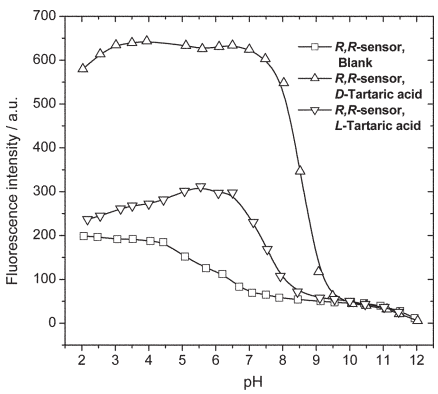


Fig. 1 Fluorescence intensity–pH profile of *R,R*-**2** with D- and L-tartaric acid; 3.0×10^{-6} mol dm^{-3} of sensor in 0.05 mol dm^{-3} sodium chloride ionic buffer (52.1% methanol), [L- and D-tartaric acid] = 0.05 M. λ_{ex} 370 nm, λ_{em} 429 nm. 22 °C.

† Electronic supplementary information (ESI) available: Selected data. pH titration curves for *S,S*-**2** and with D- and L-tartaric acid. Emission spectra of *S,S*-**2** with D- and L-tartaric acid with λ_{ex} of 370 and 275 nm at pH 2.5. See http://www.rsc.org/suppdata/cc/b4/b418279k/

*T.D.James@bath.ac.uk

Table 1 Logarithm of 1:1 stability constants, fluorescence enhancements F on binding and enantioselectivity (K_R:K_S) of sensors *R,R*-**2**, *S,S*-**2**, *R,R*-**1**, and *S,S*-**1** with D-tartaric acid[a]

	log K				F[b]				$F_R : F_S$	
pH	*R,R*-**2**	*R,R*-**1**	*S,S*-**2**	*S,S*-**1**	*R,R*-**2**	*R,R*-**1**	*S,S*-**2**	*S,S*-**1**	**2**	**1**
2.5	3.92	—[c]	2.41	—[c]	3.31	—[c]	1.49	—[c]	2.2 : 1	—[c]
5.6	5.78	5.92	4.20	4.00	9.05	4.80	3.61	3.68	2.5 : 1	1.3 : 1
7.0	4.56	4.79	2.50	2.09	13.4	9.14	5.01	8.02	2.7 : 1	1.1 : 1

[a] 3.0×10^{-6} mol dm^{-3} **1** or **2** in 0.05 mol dm^{-3} NaCl ionic buffer (52.1% methanol in water). For sensor **2**, λ_{ex} = 370 nm, λ_{em} = 429 nm; for sensor **1**, λ_{ex} = 365 nm, λ_{em} = 429 nm. 22 ± 1 °C. Constants determined by fitting a 1:1 binding model to I/I_0. Errors reported are two standard deviations (95% confidence limit); r^2 = 0.99 in most cases. [b] Determined by 1:1 fitting. F values agree well with the experimental results. [c] Due to small changes in fluorescence accurate values could not be determined.

photoinduced electron transfer (PET) can not be involved in the modulation of the fluorescent properties. With sensors *R,R*-**1** and *S,S*-**1** at pH 2.5 no fluorescence change was observed. However, with *R,R*-**2** and *S,S*-**2** the fluorescence intensity was enhanced and hence the stability of the D-tartaric acid complexes formed with *R,R*-**2** and *S,S*-**2** at pH 2.5 could be determined. At pH 5.6 and 7.0, fluorescence enhancement with both sensor types **1** or **2** is observed. With *R,R*-**2** producing the largest fluorescence response to D-tartaric acid.

In order to confirm that the complex between *R,R*-**2** and D-tartaric acid was of 1:1 stoichiometry we performed a continuous variation Job plot at pH 5.6 (Fig. 2). From the Job plot of Fig. 2 a maximum is observed at 50% indicating that *R,R*-**2** forms a 1:1 complex with D-tartaric acid.

The spectra obtained for the addition of D-tartaric acid to *R,R*-**2** can be used to help understand the fluorescence properties (Figs. 3 and 4). Addition of D-tartaric acid to *R,R*-**2** at pH 2.5 enhances the fluorescence of the anthracene fluorophore (λ_{ex} = 370 nm). Whilst addition of L-tartaric acid to *R,R*-**2** causes minimal fluorescence change (Fig. 3). Since PET can not be the cause of these fluorescence changes, the fluorescence properties indicate that D-tartaric acid causes a separation of the naphthalene and anthracene fluorophores of *R,R*-**2**. While with L-tartaric acid the two fluorophores remain in close contact and the fluorescence remains quenched. Titrations with *S,S*-**2** mirror those observed with *R,R*-**2**.

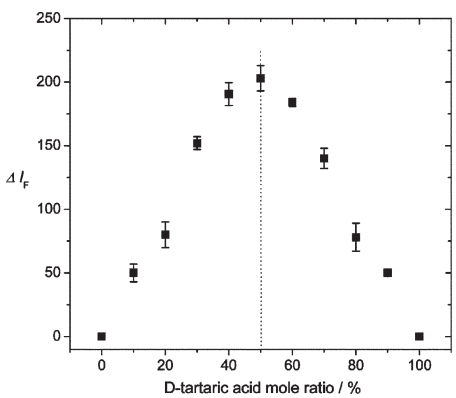


Fig. 2 Job plot of *R,R*-**2** with D-tartaric acid at a constant total concentration c (D-tartaric acid) + c (*R,R*-**2**) of 3.0×10^{-6} mol dm^{-3}; λ_{ex} = 370 nm, λ_{em} = 429 nm, pH 5.6. 5.0×10^{-2} mol dm^{-3} NaCl ionic buffer (52.1% methanol in water). 23 °C.

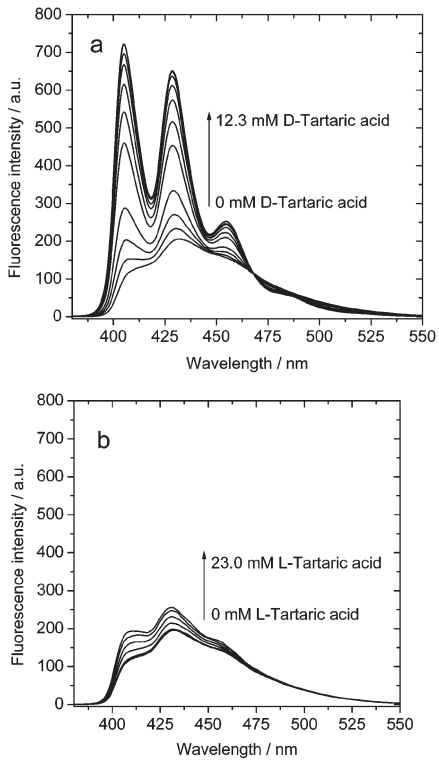


Fig. 3 Emission spectra of *R,R*-**2** in the presence of tartaric acid. a), *R,R*-**2** with D-tartaric acid; b), *R,R*-**2** with L-tartaric acid, λ_{ex} at 370 nm, pH 2.5. 3.0×10^{-6} mol dm^{-3} of sensors in 5.0×10^{-2} mol dm^{-3} NaCl ionic buffer (52.1% methanol in water). 18 °C.

When the system is excited at 275 nm (naphthalene) rather than 370 nm (anthracene), again the fluorescence of *R,R*-**2** is changed dramatically by the addition of D-tartaric acid and changed only slightly by L-tartaric acid (Fig. 4). Upon addition of L-tartaric acid the fluorescence intensity of a broad unstructured band at 440 nm decreases slightly. Addition of D-tartaric acid causes a significant change in the observed fluorescence. The broad band at 440 nm is replaced by a structured band due to emission from the anthracene fluorophore. Clearly the unstructured band at 440 nm is due to emission from the napthylene–anthracene excimer which is formed a result of the close contact between the naphthalene and anthracene fluorophores. On addition of D-tartaric acid to *R,R*-**2** the two fluorophores become separated and hence FRET from the naphthalene fluorophore to the anthracene fluorophore occurs and emission from

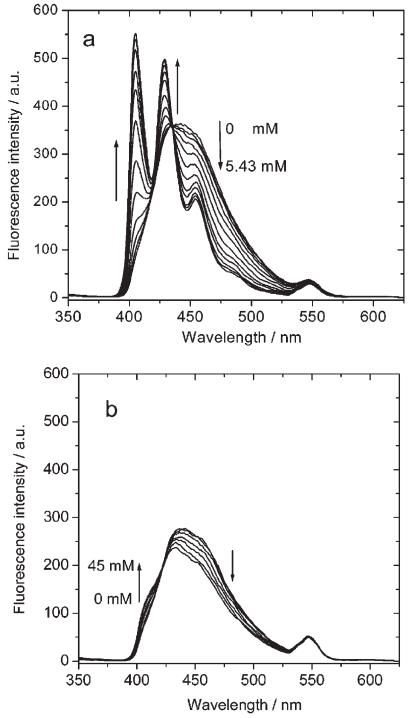


Fig. 4 Emission spectra of *R,R*-**2** in the presence of tartaric acid. a), *R,R*-**2** with D-tartaric acid; b), *R,R*-**2** with L-tartaric acid, λ_{ex} at 275 nm, pH 2.5. 3.0×10^{-6} mol dm^{-3} of sensors in 5.0×10^{-2} mol dm^{-3} NaCl ionic buffer (52.1% methanol in water). 23 °C.

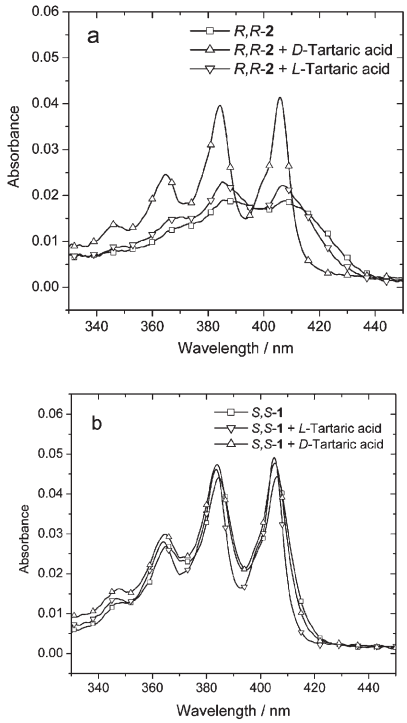


Fig. 5 Absorption spectra of *R,R*-**2** and *S,S*-**1** in the presence and absence of tartaric acid. a), *R,R*-**2**; b), *S,S*-**1**, pH 2.5. 3.0×10^{-6} mol dm^{-3} of sensors in 5.0×10^{-2} mol dm^{-3} NaCl ionic buffer (52.1% methanol in water). 23 °C.

anthracene is observed. Titrations with *S,S*-**2** mirror those observed with *R,R*-**2**.

From Fig. 5 it is clear that differences in the fluorescence properties at pH 2.5 are the result of interactions between the naphthalene and anthracene fluorophores. The UV-spectrum of *R,R*-**2** is broad indicating that the naphthalene and anthracene fluorophores are in close contact. Addition of D-tartaric acid to *R,R*-**2** produces a sharp UV-spectrum indicating separation of the two fluorophores while addition of L-tartaric acid causes minimal change in the UV-spectrum. Also, from Fig. 4 changes in the UV-spectrum of *R,R*-**1** are much less than those observed with *R,R*-**2**, indicating that interactions between benzene and anthracene in *R,R*-**1** are less than those between naphthalene and anthracene in *R,R*-**2**.

In conclusion we have shown that it is possible to enhance the signal output from a chiral discriminating fluorescent sensor by introducing an additional fluorophore. The dual fluorophore boronic acid receptor *R,R*-**2** shows an enhanced fluorescence response for D-tartaric acid, while *S,S*-**2** shows an enhanced fluorescence response for L-tartaric acid.

TDJ and JZ wish to thank Beckman-Coulter and the University of Bath for support.

Jianzhang Zhao and Tony D. James*
Department of Chemistry, University of Bath, Bath, UK BA2 7AY. E-mail: T.D.James@bath.ac.uk; Fax: +44 1225 386231; Tel: +44 1225 383810

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