Enantioselective Sensors

Chiral Binol–Bisboronic Acid as Fluorescence Sensor for Sugar Acids**

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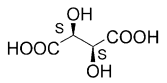
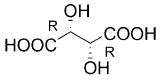
The chemistry of saccharides is of paramount importance to a wealth of biological functions within nature. By providing the building blocks for processes ranging from the production of metabolic energy to tissue recognition, saccharides have found themselves to be the focus of a vast body of research aimed at understanding and mimicking their specific role and function at a cellular level.[1] Unsurprisingly the ability to derive synthetic receptors with the capacity to selectively detect specific saccharides (and related molecules) and signal their presence by altering the optical signature has captured the attention of supramolecular chemists.[2–16]

Binol is an important chiral building block and has been widely used in asymmetric catalysis,[17,18] and for enantioselective fluorescence sensors bearing a variety of recognition elements.[19–30] In early work, a binol-based bisboronic acid (**1**) was used to recognize the enantiomers of monosaccharides, such as D/L-glucose, -fructose, and -galactose.[2,14,15] Recently



D-tartaric acid



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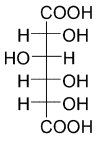

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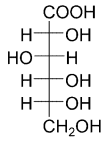

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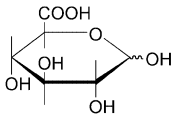

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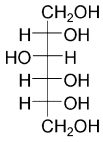
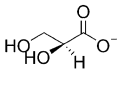
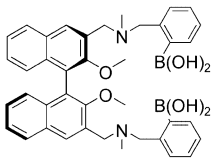


D-sorbitol



L-glycerate



R-**1**

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Houston and Gray demonstrated that racemic tartaric acid and other α-hydroxy acids can bind with the racemic form of **1**.[31] The binding constant reported for tartaric acid ($1.41 \times 10^3\,\text{M}^{-1}$ at pH 7.77) is comparable to the strengths of interactions with monosaccharides.[2,14,15] Boronic-acid-based fluorescence sensors for tartaric acid,[32] D-glucuronate,[33,34] and D-glucarate[35] have been reported, as has a boronic-acid-based colorimetric indicator-displacement assay for the determination of the enantiomeric excess of α-hydroxy acids.[36] Hydrogen-bonding receptors for the binding of tartaric acid[37] and the chiral discrimination of hydroxy carboxylates[38] and tartaric acid[39,40] are also known.

In order to develop improved fluorescent chiral discriminating systems, we investigated the interaction of the two chiral forms of sensor **1** (*R* and *S*) with a range of sugar acids over a range of pH values. The results for titrations of *R*-**1** and *S*-**1** with D- and L-tartaric acid are given in Figure 1.

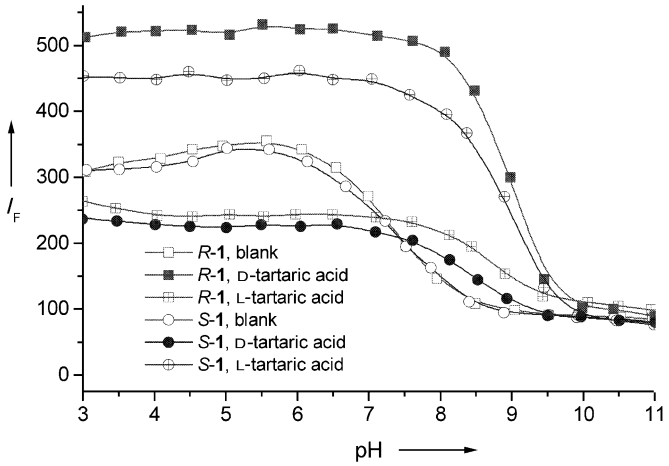

Figure 1. Fluorescence intensity–pH profiles for titrations of *R*- and *S*-**1** with D- or L-tartaric acid. 5.0×10^{-6} mol dm^{-3} **1** in 0.05 mol dm^{-3} NaCl solution (52.1% methanol in water), c(D-tartaric acid) = c(L-tartaric acid) = 0.02 mol dm^{-3}, λ_{ex} = 289, λ_{em} = 358 nm, 23 °C.

The recognition of D- and L- tartaric acid by sensor **1** (*R* or *S*) is strongly pH dependent. The fluorescence enhancement for the complexes formed by either *R*- or *S*-**1** at pH 8.3 is expected.[2,14,15] Remarkably, at pH 5.6 the fluorescence of *S*-**1** is *enhanced* by the presence of L-tartaric acid, but for *R*-**1** with the same analyte, the fluorescence is *diminished* relative to that of the free bisboronic acid. Conversely, the fluorescence of the sensor *R*-**1** is *enhanced* by the presence of D-tartaric acid, but for S-**1** with D-tartaric acid, the fluorescence is *diminished* relative to that of the free bisboronic acid. The fluorescence of the binol moiety can be enantioselectively diminished (via a non-emissive exiplex, or by charge-transfer between the analyte and the sensor),[19–21] or enantioselectively enhanced (usually by photoinduced electron transfer (PET)).[2,14,15,19,27,29] However, previous reports of chiral discrimination were based solely on a different *extent* of the spectral change in a single sense (either enhanced or diminished),[19] rather than on the *sense* of the change.

Fluorescence intensity–pH profiles for titrations with all four saccharides (glucaric acid, gluconic acid, glucuronic acid, and sorbitol) showed that they were bound by *R*/*S*-**1**. However, only D-gluconic acid showed the same type of behavior as the tartrates: at pH 5.6 the signal of the D-gluconate complex with *S*-**1** was augmented relative to that of the starting bisboronate whereas for *R*-**1** the signal was diminished. This implies that the normal PET quenching that gives rise to the fluorescence changes must be mediated by geometrical changes in individual sensor–analyte complexes.[19,41] Static quenching within a complex is more likely than dynamic quenching, both on these steric grounds, and because the effect occurs at very low analyte concentration due to the high binding constants (see below).[19,41]

Binding constants were determined at pH 5.6 and 8.3 where a 1:1 binding isotherm was fit to the normalized fluorescence (I/I_0) as function of added substrate (Figure 2, Table 1).[42] The values for the tartaric acid complexes at pH 8.3 are of the same order of magnitude as those reported for the racemic system,[31] but at pH 5.6 the apparent

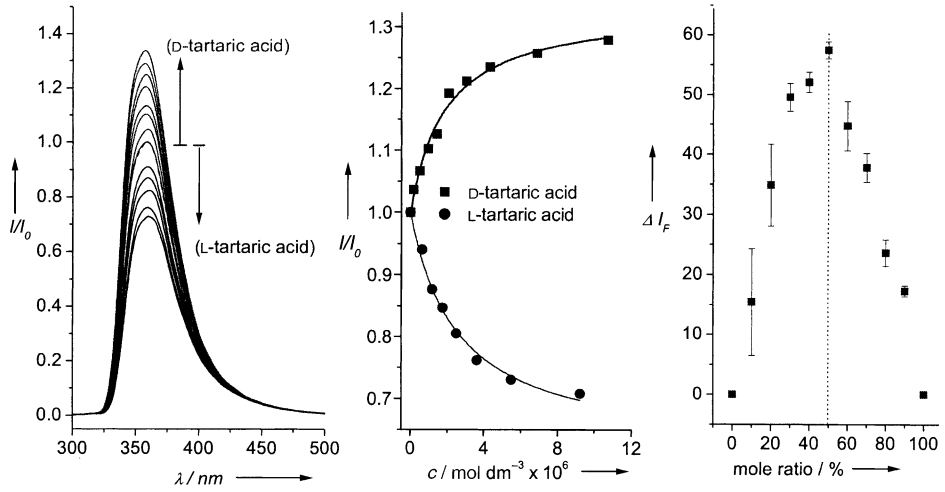

Figure 2. Chiral discrimination of D- and L-tartaric acid by *R*-**1** at pH 5.6. c(**1**) = 5.0×10^{-6} mol dm^{-3}, pH 5.6 in 0.05 mol dm^{-3} NaCl (52.1% methanol in water), λ_{ex} = 289 nm, 22 °C. The pH was kept at 5.6 with NaOH/HCl. Left: emission spectra. Center: normalized emission intensity as a function of the concentration of added tartaric acid. Lines represent the fit of a 1:1 binding isotherm. Right: Job plot of *R*-**1** with D-tartaric acid at a constant total concentration c(D-tartaric acid) + c(*R*-**1**) of 5.0×10^{-6} mol dm^{-3}; λ_{em} = 358 nm.

Table 1: Logarithm of 1:1 stability constants *K*, fluorescence enhancement *F* on binding, and response enantioselectivity of sensors *R*-**1** and *S*-**1**.[a]

Analyte	pH	lg *K*		*F*		Response selectivity[b]
		R-**1**	*S*-**1**	*R*-**1**	*S*-**1**	*R*:*S*
D-tartaric acid	5.6	5.72 ± 0.12	5.42 ± 0.16	1.39 ± 0.04	0.53 ± 0.01	(5.2 ± 2.5):1
	8.3	3.71 ± 0.06	2.21 ± 0.12	3.09 ± 0.11	1.37 ± 0.04	(71 ± 23):1
L-tartaric acid	5.6	5.59 ± 0.12	5.44 ± 0.10	0.62 ± 0.05	1.47 ± 0.01	1:(3.3 ± 1.2)
	8.3	2.02 ± 0.12	3.37 ± 0.06	1.37 ± 0.02	2.59 ± 0.06	1:(42 ± 13)
D-gluconic acid	5.6	4.45 ± 0.18	5.42 ± 0.26	0.81 ± 0.01	1.22 ± 0.01	1:(14 ± 5)
	8.3	3.61 ± 0.06	4.38 ± 0.08	2.31 ± 0.04	3.10 ± 0.14	1:(8 ± 2)
D-glucaric acid	8.3	4.47 ± 0.14	4.11 ± 0.06	2.32 ± 0.11	3.08 ± 0.10	(1.7 ± 0.6):1
D-glucuronic acid	8.3	3.52 ± 0.14	3.55 ± 0.14	3.43 ± 0.05	2.63 ± 0.03	(1.2 ± 0.6):1
D-sorbitol[c]	8.3	4.13 ± 0.06	4.16 ± 0.04	3.23 ± 0.01	2.91 ± 0.01	(1.0 ± 0.2):1

[a] 5.0×10^{-6} mol dm^{-3} *R*-**1** or *S*-**1** in 0.05 mol dm^{-3} NaCl solution (52.1% methanol in water), $\lambda_{ex}=289$, $\lambda_{em}=358$ nm, (23 ± 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 all cases. [b] Response selectivity = $(K(R)\,F(R))/(K(S)\,F(S))$. [c] Values determined from a sequential 1:1 plus 2:1 binding model. $\lg K_2(R\text{-}\mathbf{1}) = 1.80\pm0.10$, $F=1.6\pm0.02$; $\lg K_2(S\text{-}\mathbf{1})=1.71\pm0.08$, $F=1.59\pm0.02$.

constants are about 100-fold greater. This is presumably due to nitrogen protonation which would provide an adjacent positive charge to stabilize the boronate. Table 1 also reports a derived response-selectivity factor: the product of the chiral selectivity of binding (K_R/K_S) and the chiral selectivity of the fluorescence enhancement ($F(R)/F(S)$). The overall range is large—ranging from indiscriminate (with D-sorbitol) up to an approximately 50-fold difference between *R*- and *S*-**1** with the tartaric acids at pH 8.3.

The opposing effect of enantiomeric D- and L-tartaric acid on the fluorescence of *R*- and *S*-**1** allows the system to be used to determine the enantiomeric excess of a D/L tartaric acid mixture (Figure 3). The data can be related to the relative

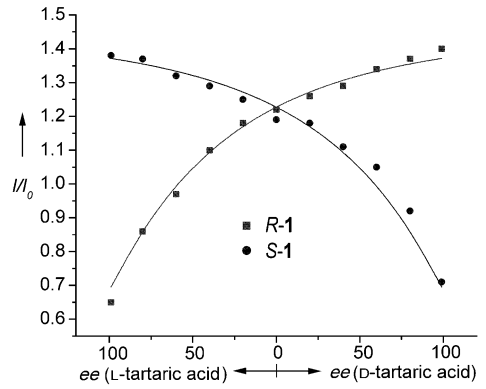

Figure 3. Relative fluorescence changes for *R*- and *S*-**1** in mixtures of D- and L-tartaric acid. $c(R\text{-}\mathbf{1})$ or $c(S\text{-}\mathbf{1}) = 5\times10^{-6}$ mol dm^{-3}, c(D-tartaric acid) + c(L-tartaric acid) = 0.01 mol dm^{-3}, pH 5.6 in 0.05 mol dm^{-3} NaCl (52.1% methanol in water), $\lambda_{ex}=289$, $\lambda^{em}=358$ nm, 22 °C.

binding constants for the diasteromeric complexes and to the enhancement factors for the fluorescence of these complexes. A model that considers only 1:1 complexes cannot reproduce the shape of the curves. However, at the total tartaric acid concentration used for Figure 3, the extent of 2:1 binding is significant (see Supporting Information). We therefore considered a model of mixed 1:1 and 2:1 complexes in which the properties of the enantiomeric components were assumed to be equal.

In this model, there are four binding constants and four enhancement factors. Some of these parameters are known independently (Table 1) and likely values for the enhancement factors for the 2:1 complexes are evident from the titrations done to high concentration. The model was fit to the combined data of Figure 3 to refine these eight parameters and to produce the curves shown. The values determined by this method are: $\lg K_1(R_L) = \lg K_1(S_D) = 5.33\pm0.1$, $\lg K_1(R_D) = \lg K_1(S_L) = 5.84\pm0.1$, $\lg K_2(R_{L_2}) = \lg K_2(S_{D_2}) = 0.9\pm0.2$ and $\lg K_2(R_{D_2}) = \lg K_2(S_{L_2}) = 1.2\pm0.2$; $F_1(R_L) = F_1(S_D) = 0.63\pm0.03$, $F_1(R_D) = F_1(S_L) = 1.42\pm0.02$, $F_2(R_{L_2}) = F_2(S_{D_2}) = 1.2\pm0.3$ and $F_2(R_{D_2}) = F_2(S_{L_2}) = 1.2\pm0.3$ ($K_1(R_D)$ is the binding constant for the 1:1 complex of *R*-**1** and D-tartaric acid; $F_2(R_{L_2})$ is the enhancement factor for the 1:2 complex of *R*-**1** and L-tartaric acid). The same values correctly reproduce the individual titration curves (see Supporting Information). A further confirmation of the model is that the 1:1 binding constants and enhancement factors determined from Figure 3 data agree within the experimental uncertainty with the independently determined values for the same complexes in Table 1.

The data in Table 1 are consistent with the structural proposals of previous work in which "bridging" of the two boronic acid sites by the bound substrate led to stable 1:1 complexes.[2,14,15] Binding of L-glycerate by **1** (*R* or *S*) was not observed presumably because this substrate lacks a second binding site for bridging. Similarly, the cyclic substrate D-glucuronic acid has smaller binding constants than the linear D-glucaric acid. Linear saccharic acids can adjust to be complementary to the binding requirement of the sensor, and therefore show generally higher binding constants. The chiral discrimination in binding is independent of the chiral discrimination in fluorescence enhancement. The former varies to a larger extent (up to about 25-fold) than does the latter (up to about 3-fold), so the most significant factor determining the overall response selectivity is the binding selectivity. Geometrical differences thus play a larger role in substrate recognition than they do in the photophysical processes that lead to the signal.

In summary, our study shows that the binol-based sensor **1** can selectively bind a range of saccharic acids, and that binding with some analytes is highly enantioselective. Moreover, a novel fluorescence response to enantiomeric analytes has been observed. We believe that these results will offer new ideas for the analysis of these metabolic intermediates with synthetic fluorescence sensors. Our future work will focus on the design of binol-based sensors with enhanced

binding selectivity towards sugar acids, in the expectation that these will lead to even higher response selectivities.

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Keywords: boronic acids · chiral recognition · fluorescence sensors · sugar acids · tartaric acid

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[42] At low concentrations, the binding of the saccharides in Table 1 occurs with a 1:1 stoichiometry. This is implied by the excellent fits of the model, and is more directly demonstrated by the Job plot given in Figure 2 (right) for the *R*-**1** plus D-tartrate system. However, at higher concentrations (> millimolar), all systems show some deviation from the expectations of a strict 1:1 binding model. The sorbitol system clearly indicates that these deviations are due to 2:1 complexes. For further discussion see the Supporting Information.

