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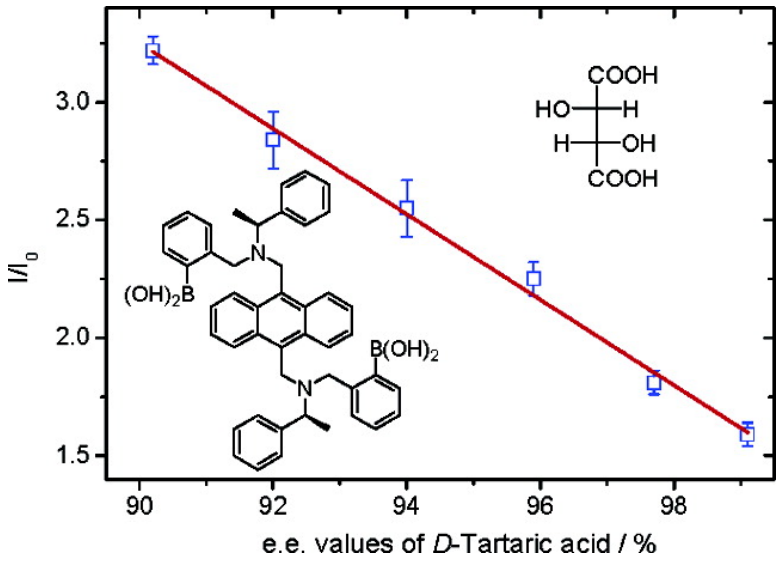

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An Enantioselective Fluorescent Sensor for Sugar Acids

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Abstract: Chiral fluorescent boronic acid **1** was found to be a highly enantioselective, chemoselective, and sensitive sensor for sugar acids, such as tartaric acid. Enantioselectivities (K_R/K_S) of up to 550:1, chemoselectivity up to 11 000:1, and sensitivities in the micromolar range with sensor **1** were observed. Single-crystal X-ray analysis was used to confirm the structure of the fluorescent species.

Introduction

Much recent attention has been paid to the development of synthetic molecular receptors with the ability to recognize 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] Boronic acid molecular receptors for saccharides have attracted considerable interest due to their ability to bind saccharides in aqueous media.[3−6] In this case, the most common interaction is with *cis*-1,2- or 1,3-saccharide diols to form five- or six-membered rings with the boronic acid moiety. Over the past few years we have been interested in developing new selective sensors for saccharides employing a modular approach.[7−12] Our idea was to disconnect a sensor into three tuneable components: receptor, linker, and "read-out" units. This approach can be illustrated by the D-glucose-selective fluorescent sensor **3** (Scheme 1) which contains two boronic acid units (binding units) and an anthracene unit (acting as both a D-glucose selective linker and the fluorophore read-out unit).[13,14]

Fluorescent boronic acid-based sensors for tartaric acid,[15,16] D-glucuronic acid,[17,18] and D-glucaric acid[19] have been reported, as has a boronic acid-based colorimetric indicator-displacement assay for the determination of enantiomeric excess of α-hydroxy acids.[20] Hydrogen-bonding receptors for the binding of tartaric acid,[21] chiral discrimination of hydroxylcarboxylates,[22] and tartaric acid[23,24] are also known.

Herein we report the tight and selective binding of the sugar acids, D- and L-tartaric acid, D-glucaric acid, and D-gluconic acid with the rationally designed chiral fluorescent sensors **1** and **2**.

Most naturally occurring chemical species, including sugars and sugar acids, are chiral compounds; therefore, a chiral sensor is inherently better than an achiral one, since the chirality of the analytes can be utilized as an extra differential factor to enhance discrimination. In an attempt to address this need for enantioselectivity we recently employed BINOL. The rigid axial chirality of BINOL was key in the selective chiral sensor's construction (functioning as chirogenic center and fluorophore).[25] 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 sensor **1** which has two chiral centers in close proximity to the binding site of the receptor and used anthracene, a good fluorophore, as a rigid linker.

Results and Discussion

The fluorescence response of the boronic acid sensor as well as the binding of the carboxylic moiety, is pH-dependent; therefore, the fluorescence−pH profiles of sensor **1** in the

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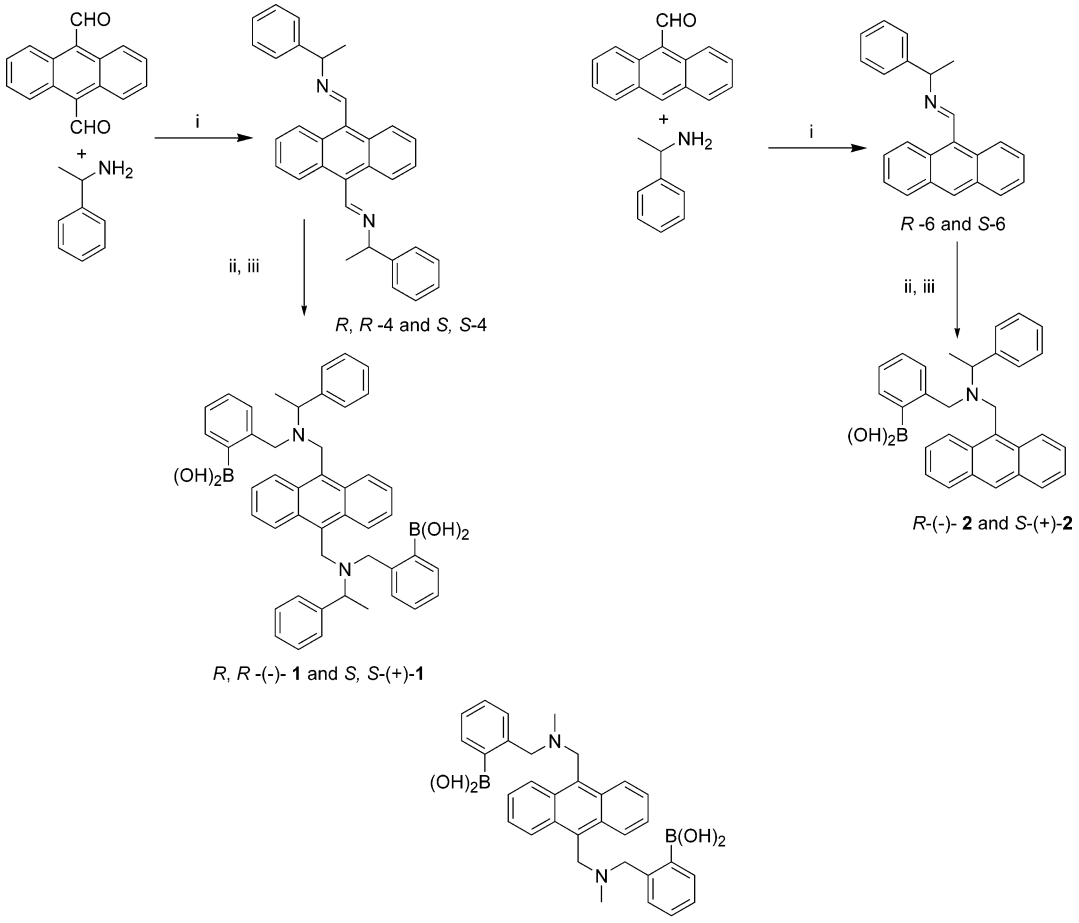
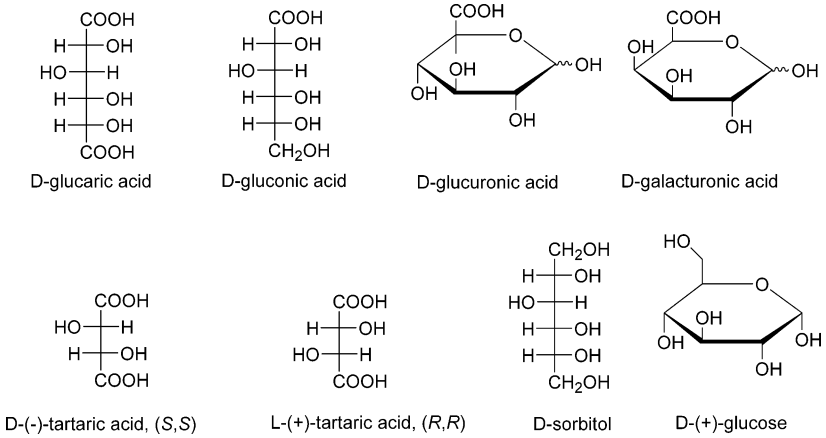
Scheme 1. Fluorescent Sensors for Monosaccharides or Sugar Acids[a]



[a] Reagents and conditions: i, EtOH/THF (7:2), reflux, 6 h, 76.8% (*R*,*R*-**4**), 76.8% (*S*,*S*-**4**), 85.2% (*R*-**6**), 90.0% (*S*-**6**); ii, THF, $NaBH_4$, 60.1% (*R*,*R*-**5**), 78.9% (*S*,*S*-**5**), 85.2% (*R*-**7**), 81.1% (*S*-**7**); iii, CH_3CN, 2-(2-bromobenzyl)-1,3-dioxaborinane, reflux, 10 h, 66.4% (*R*,*R*-**1**)0.78.5% (*S*,*S*-**1**), 14.0% (*R*-**2**), 5.2% (*S*-**2**).

Scheme 2. Analytes Used in the Study



presence of various analytes (Scheme 2) were determined since this allows a rapid preview of the optimal pH region of the sensor. That is, the appropriate pH value that will result in large fluorescence changes. The results observed for sensor **1** with D- and L-tartaric acid are shown in Figure 1a. In the presence of D- and L-tartaric acid, sensor *R*,*R*-**1** (or *S*,*S*-**1**) gave different responses (Figure 1a). The apparent pK_a value of *S*,*S*-**1** is 4.82 ± 0.04 and in the presence of D- or L-tartaric acid, this shifts to 7.62 ± 0.04 or 8.73 ± 0.04, respectively. While for *R*,*R*-**1**, the pK_a value of 4.81 ± 0.05 changes to 8.66 ± 0.05 or 7.61 ± 0.05 respectively, in the presence of D- or L-tartaric acid (Figure 1a). The complementarities of the pK_a values between enantiomers of sensor **1** and tartaric acid clearly demonstrate enantioselective recognition by the boronic acid sensors for tartaric acid. With the monoboronic acid sensor **2**, however, no enantioselectivity was observed (Figure 1b). The apparent pK_a value of *R*-**2** is 5.91 ± 0.02, and in the presence of D- and L-tartaric acid this shifts to 8.04 ± 0.02 and 8.14 ± 0.01,

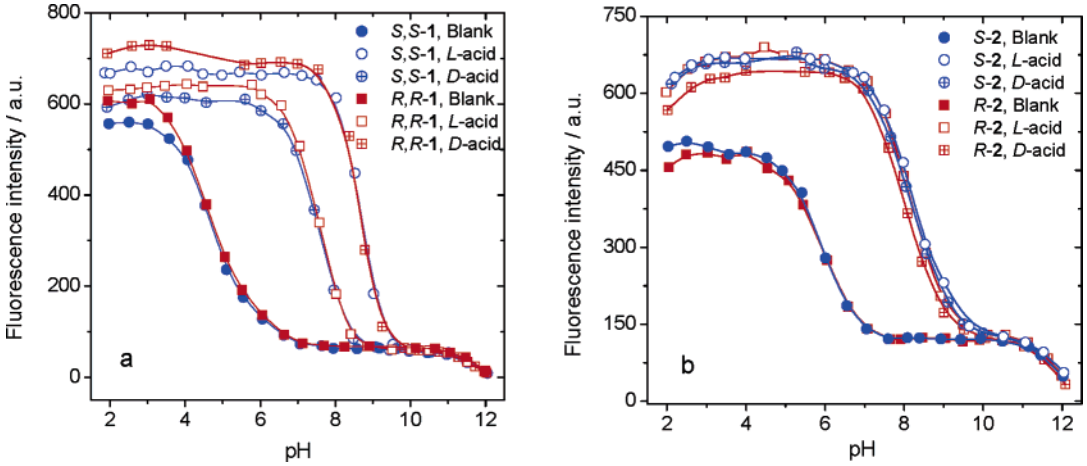


Figure 1. Fluorescence intensity−pH profile of sensors **1** and **2**. (a) Bisboronic acid **1** with D- and L-tartaric acid, λ_{ex} at 365 nm, λ_{em} at 429 nm. (b) Monoboronic acid **2** with D- and L-tartaric acid, λ_{ex} at 373 nm, λ_{em} at 421 nm. 3.0×10^{-6} mol dm^{-3} of sensors in 5.0×10^{-2} mol dm^{-3} NaCl ionic buffer (52.1% methanol in water), [L- and D-tartaric acid] = 5.0×10^{-2} mol dm^{-3}, 22 °C.

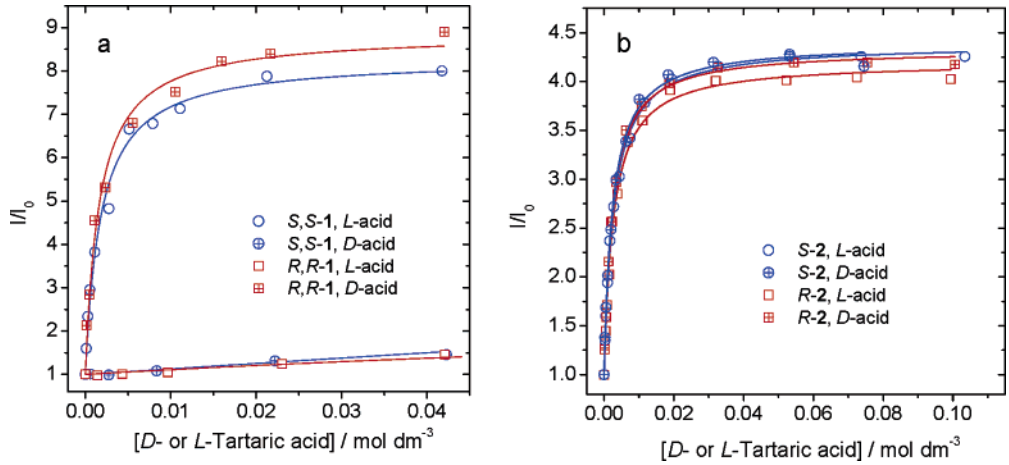


Figure 2. Relative fluorescence intensity of sensors **1** and **2** vs concentration of D- or L-tartaric acid. (a) Bisboronic acid **1** with D- and L-tartaric acid, λ_{ex} at 365 nm, λ_{em} at 429 nm, pH 8.3. (b) Monoboronic acid **2** with D- and L-tartaric acid, λ_{ex} at 373 nm, λ_{em} at 421 nm, pH 7.0. 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), 22 °C.

respectively. The pK_a of *S*-**2** is 5.89 ± 0.05, and in the presence of D- and L-tartaric acid this shifts to 8.17 ± 0.06 and 8.23 ± 0.06, respectively.

Titrations of the sensors with tartaric acid were carried out at pH 8.3, 7.0, and 5.6. At pH 8.3 (Figures 2 and 3) a significant fluorescence enhancement was observed with *S*,*S*-**1** ($I/I_0 = 8.24$) in the presence of L-tartaric acid (Figures 2a and 3a), whereas in the presence of D-tartaric acid (Figures 2a and 3b), only a very small fluorescence enhancement was observed ($I/I_0 = 1.5$). When *R*,*R*-**1** was used, the fluorescence response mirrored that observed with *S*,*S*-**1**, in that a large fluorescence enhancement ($I/I_0 = 8.87$) was observed with D-tartaric acid (Figure 2a), whereas in the presence of L-tartaric acid (Figure 2a) only a small fluorescence response was observed ($I/I_0 = 1.5$). The binding constants of the sensor with the D- or L-tartaric acid are also significantly different (Table 1). For example, the binding constants of *R*,*R*-**1** with the D-tartaric acid is log K_D 2.79 ± 0.12, while its binding constant with L-tartaric acid was not determined due to the small changes in fluorescence.

At pH 7.0 the binding constants of *R*,*R*-**1** with the D- and L-tartaric acid were log K_D 4.79 ± 0.07 and log K_L 2.07 ± 0.06, and the binding constants of *S*,*S*-**1** with the D- and L-tartaric acid were log K_D 2.09 ± 0.06 and log K_L 4.81 ± 0.06. Hence, for this system, the enantioselectivity (K_R:K_S) is 490:1 for D-tartaric acid and 1:550 for L-tartaric acid.

At pH 5.6 the enantioselectivity and relative fluorescence enhancement is diminished. However, the binding of tartaric acid with sensor **1** at pH 5.6 is 1000 times stronger than the values obtained at pH 8.3. The binding constants (log *K*) of D-tartaric acid with *R*,*R*-**1** is 5.92 ± 0.17, compared to the value of 2.79 ± 0.12 at pH 8.3. The improvement in the binding constants is significant and desirable since the detection limit can be improved from the millimolar to the micromolar range.

As the stability constants and fluorescence enhancement of sensor **1** with D- or L-tartaric acid are highly enantioselective, the ee of tartaric acid mixtures can be determined (Figure 4). Also, since the dynamic range of the fluorescence response is large the system is very sensitive. The range of ee chosen was from 90 to 100 since this is the range of interest in monitoring enantioselective reactions. The large fluorescence response with sensor **1** makes it possible to detect ee differences of only 1% (Figure 4).

The binding of the sensor **1** with other sugar acids, such as glucaric acid, gluconic acid, glucuronic acid and galacturonic acid as well as sorbitol and glucose were also investigated and the binding constants, fluorescent enhancements and enantio-

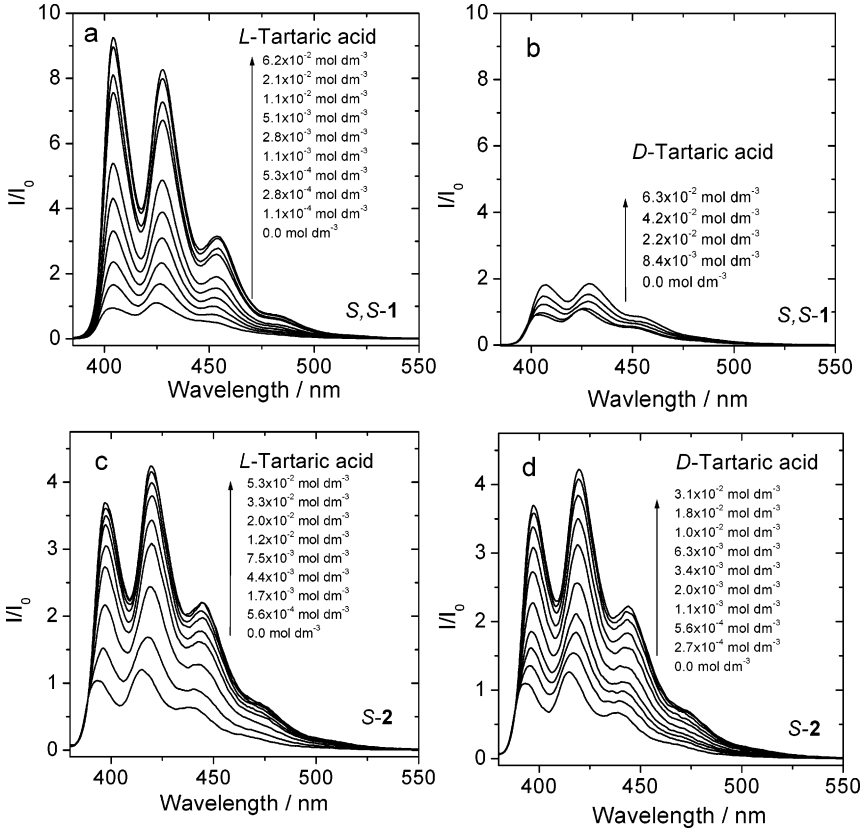


Figure 3. Normalized emission spectra of sensors *S,S*-**1** and *S*-**2** in the presence of tartaric acid. (a) *S,S*-**1** with L-tartaric acid; (b) *S,S*-**1** with D-tartaric acid, λ_{ex} at 365 nm, pH 8.3; (c) *S,S*-**2** with L-tartaric acid; (d) *S*-**2** with D-tartaric acid, λ_{ex} at 373 nm, pH 7.0. 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), 22 °C.

Table 1. Logarithm of 1:1 Stability Constants, Fluorescence Enhancement *F* on Binding, and Enantioselectivity (K_R:K_S) of Sensors *R,R*-**1**, *S,S*-**1**, *R*-**2**, and *S*-**2**[a]

		log *K*		*F*[b]		
analytes	pH	*R,R*-**1**	*S,S*-**1**	*R,R*-**1**	*S,S*-**1**	K_R:K_S
D-tartaric acid	5.6	5.92 ± 0.17	4.00 ± 0.11	4.80 ± 0.12	3.68 ± 0.06	83:1
	7.0	4.79 ± 0.07	2.09 ±0.06	9.14 ± 0.15	8.02 ± 0.14	490:1
	8.3	2.79 ± 0.12	–[c]	8.87 ± 0.22	1.5[d]	–
L-tartaric acid	5.6	3.93 ± 0.13	5.76 ± 0.16	3.71 ± 0.06	4.36 ± 0.14	1:67
	7.0	2.07 ± 0.06	4.81 ± 0.06	8.03 ± 0.12	9.42 ± 0.10	1:550
	8.3	–[c]	2.74 ± 0.10	1.5[d]	8.24 ± 0.21	–
DL-tartaric acid	7.0	4.68 ± 0.08	4.71 ± 0.08	8.61 ± 0.12	8.33 ± 0.11	1:1.1
D-glucaric acid[e]	5.6	4.76 ± 0.40	5.73 ± 0.13	4.80 ± 0.06	5.52 ± 0.11	1:9.2
	8.3	2.31 ± 0.20	2.68 ± 0.07	2.96 ± 0.15	9.24 ± 0.19	1:2.3
D-gluconic acid[f]	5.6	4.09 ± 0.07	4.73 ± 0.04	3.77 ± 0.04	4.08 ± 0.03	1:4.4
	8.3	2.41 ± 0.15	2.93 ± 0.05	3.51 ± 0.12	7.62 ± 0.13	1:3.3
D-glucuronic acid	5.6	3.52 ± 0.05	3.81 ± 0.04	4.86 ± 0.05	5.26 ± 0.19	1:2.0
	8.3	2.86 ± 0.19	3.01 ± 0.03	3.26 ± 0.12	14.5 ± 0.89	1:1.4
D-galacturonic acid	5.6	3.02 ± 0.12	2.93 ± 0.04	4.97 ± 0.15	3.76 ± 0.04	1.2:1
D-glucose	8.3	1.45 ± 0.12	1.38 ± 0.08	14.2 ± 0.76	15.8 ± 0.58	1.2:1
D-sorbitol	5.6	1.87 ± 0.06	3.31 ± 0.09	5.07 ± 0.08	4.06 ± 0.06	1:27
	8.3	1.93 ± 0.04	3.30 ± 0.15	11.1 ± 0.13	9.51 ± 0.28	1:24
		R-**2**	*S*-**2**	*R*-**2**	*S*-**2**	
D-tartaric acid	5.6	3.47 ± 0.29	3.48 ± 0.04	1.70 ± 0.00	1.80 ± 0.01	1:1.0
	7.0	2.62 ± 0.03	2.61 ± 0.04	4.33 ± 0.03	4.40 ± 0.04	1.0:1
L-tartaric acid	5.6	3.47 ± 0.08	3.54 ± 0.08	1.75 ± 0.01	1.74 ± 0.01	1:1.2
	7.0	2.57 ± 0.04	2.57 ± 0.03	4.20 ± 0.04	4.39 ± 0.02	1.0:1

[a] 5.0×10^{-6} mol dm^{-3} *R,R*-**1** or *S,S*-**1** in 0.05 mol dm^{-3} NaCl ionic buffer (52.1% methanol in water), 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. [d] Maximum fluorescence enhancement observed. [e] The potassium glucarate salt of glucaric acid was used in the titration. [f] The sodium gluconate salt of gluconic acid was used in the titration.

selectivities (K_R:K_S) are listed in Table 1. The data in Table 1 indicates that this system is highly chemoselective for the sugar acids. For example, the chemoselectivity of *R,R*-**1** for D-glucaric acid/D-gluconic acid is 9.9:1 (pH 5.6) and for D-glucaric acid/

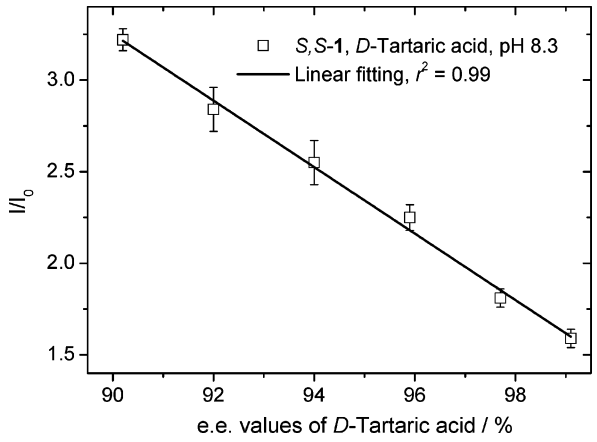


Figure 4. Fluorescence intensity changes of *S,S*-**1** vs enantiomeric composition of D-tartaric acid. 3.0×10^{-6} mol dm^{-3} of *S,S*-**1** in 5.0×10^{-2} mol dm^{-3} M NaCl ionic buffer (52.1% methanol in water), λ_{ex} 365 nm, λ_{em} 429 nm, pH 8.3, 22 °C. [D-tartaric acid] + [L-tartaric acid] = 6.0×10^{-2} mol dm^{-3}. The variation of the ee value of the D-tartaric acid was achieved with mixing the D-tartaric acid and the L-tartaric acid.

D-sorbitol is 790:1. Moreover, the chemoselectivity of sensor **1** can be optimized by changing the pH of the solution. For example, at pH 8.3, the chemoselectivity of *R,R*-**1** for D-glucaric acid/D-glucuronic acid is 1:3.5 but at pH 5.6 the selectivity changes to 18:1. With *S,S*-**1** the D-glucaric acid/D-glucuronic acid selectivity changes from 1:2.2 (pH 8.3) to 82:1 (pH 5.6).

The best selectivity improvement is observed for *R,R*-**1** and D-tartaric acid/D-sorbitol, 7.2:1 (pH 8.3) and 11 000:1 (pH 5.6). Such selectivity enhancements and in particular a switch in selectivity with pH is highly desirable since in principle it will allow the concentration of sugar acids in mixtures to be determined.

From the data in Table 1, Figures 1b, 2b, 3c, and 3d, no enantioselective discrimination between D- and L-tartaric acid was observed when the monoboronic acid sensors *R*-**2** and *S*-**2** were used instead of the diboronic acid sensors *R,R*-**1** and *S,S*-**1**. This result indicates that for enantioselective discrimination 1:1 cyclic complexes must be formed between *R,R*-**1** and *S,S*-**1** and the sugar acids. The data in Table 1 also indicate that the binding constants with the sugar acids are strongly pH-dependent whereas those of the sugar alcohols are not.

Another advantage of these sensors is in utilizing their chirality to provide chemoselectivity between the single enantiomers of two different biological molecules. For example, at pH 5.6 the chemoselectivity of *S,S*-**1** for D-glucaric acid/D-sorbitol is 260:1; however, by using *R,R*-**1**, the D-glucaric acid/D-sorbitol chemoselectivity can be increased to 790:1. With D-tartaric acid/D-sorbitol at pH 5.6 the chemoselectivity of *S,S*-**1** is 4.9:1; however, by using *R,R*-**1**, the chemoselectivity is increased to 11 000:1. With simple achiral sensors, such optimization of sensitivity and selectivity is impossible.

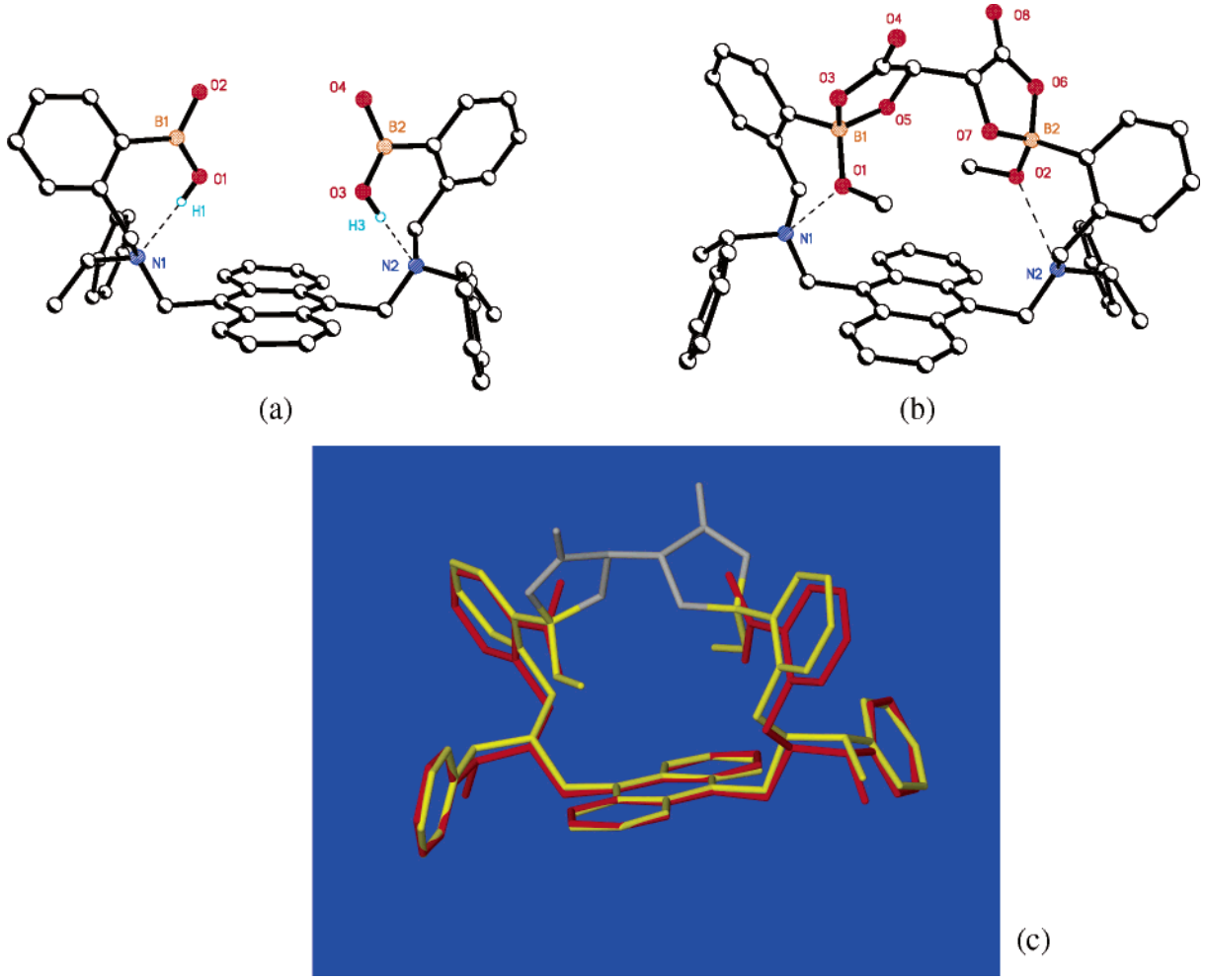


Figure 5. (a) Structure of *R,R*-**1**. (b) Structure of *S,S*-**1** complex with L-tartaric acid. (c) Overlay for comparison of structure (a) (mirror image) and structure (b).

Although a large number of boronic acid PET sensors have been studied, to our knowledge, no single-crystal X-ray data for a fluorescent diboronic acid sensor bound with a guest have been reported until now. With sensor **1**, the structure of the 1:1 binding complex with tartaric acid was determined, as was the structure of the unbound receptor (Figure 5). For the unbound receptor, the hydrogen atoms of the boronic acid were located and participate in O−H···N hydrogen bonds with the nitrogen atoms of the receptor framework (Figure 5a: N(1)···O(1), 2.764 Å; N(2)···O(3), 2.759 Å). For the tartrate complex (Figure 5b) a methanol (CH_3OH) is bound through its oxygen atom to the boron center and also hydrogen bonds to the nitrogen atom of the receptor framework (N(1)···O(1), 2.655 Å; N(2)···O(2), 2.693 Å). In this latter case the hydrogen atoms of the methanol could not be located and refined, but their position is inferred by the similarity in geometry between the unbound and bound receptors (Figure 5c). These structural data clearly demonstrate that binding of the sugar acid to the boronic acid does not result in the formation of a strong B−N bond (the molecular property believed to account for the fluorescence enhancement in this type of sensor). These structural observations agree with a recent computational study which indicated that the B−N bond was weak (13 kJ mol^{-1} in the absence of solvent).[26]

Experimental Section

General. All the solvents and chemicals were supplied by Frontier Scientific Ltd., Aldrich Chemical Co. Ltd., Lancaster Synthesis Ltd., and Fisher Scientific Ltd. Dry solvents for synthesis were freshly distilled over suitable drying agents prior to use. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 300 (300.13 and 75.47 MHz respectively) spectrometer. All the chemical shifts (δ) are reported in ppm using the deuterated solvent and or tetramethylsilane as the internal reference. The mass spectra were recorded on a Waters Micromass Autospec Spectrometer using fast atom bombardment (liquid secondary ion mass spectrometry) using 3-nitrobenzyl alcohol (NOBA) as the matrix liquid. Elemental Analyses were performed on an Exeter Analytical CE 440. Thin-layer chromatography (TLC) was performed on precoated aluminum-backed silica or alumina plates supplied by Fluka Chemie. Visualization was achieved by UV light (254 nm). The specific rotation of the chiral sensors ($[\alpha]^{22}_D$) was measured with a AA-10 automatic polarimeter (Optical Activity Ltd., England) using a 10 cm cell.

Fluorescence spectra were measured on a Perkin-Elmer LS 50B luminescence spectrometer. Data were collected and exported via the Pekin Elmer FL WinLab 4.00 software package. The fluorescence intensity changes of bisboronic acid **1** was monitored at 429 nm (λ_{ex} 365 nm). For monoboronic acid **2**, the fluorescence was monitored at 421 nm (λ_{ex} 373 nm). A 0.05 mol dm^{-3} NaCl (52.1% methanol, w/w) ionic buffer was used in all the experiments. The final concentrations of the sensors were fixed at 5.0×10^{-6} mol dm^{-3} (by dilution of the stock solution into the buffer). All pH measurements were recorded on a Hanna Instruments HI 9321 Microprocessor pH meter which was routinely calibrated using Fisher Chemicals standard buffer solutions (pH 4.0 for phthalate, 7.0 for phosphate, and 10.0 for borate).

pH Titration. The fluorescence emission spectra of the sensors with or without the analytes were recorded as the pH was changed from pH 2 to 12 in approximate intervals of 0.5 pH unit. The pH of the solution was controlled using minimum amount of sodium hydroxide and hydrochloric acid solutions. For every concentration or pH data point the fluorescence was recorded three times and the mean values were used for the final curves.

Titration with Analytes. The fluorescence spectra of the sensors in the presence of analytes were recorded as increasing amounts of analytes were added to the solution. For all titrations the final pH was controlled to within less than ±0.03 unit from the desired pH. Titration plots were generated using Origin 5.0 (Microcal software). The binding constants were calculated using custom-written nonlinear least-squares curve-fitting programs implemented within SigmaPlot 2000 (SPSS Inc.).

Single-Crystal Preparation and Crystallographic Methods. The *R,R*-**1** single crystal was obtained by dissolving the compound in dichloromethane/methanol mixture (5:1) to give a concentrated solution. Good quality crystals were obtained within about 2 weeks. The *S,S*-**1** with L-tartaric acid single crystal was obtained by the two layer diffusion method (sensor *S,S*-**1** was dissolved in dichloromethane and the L-tartaric acid was dissolved in methanol).

Crystallographic data for *R,R*-**1** and *S,S*-**1** with L-tartaric acid are available as Supporting Information. Data were collected on a Nonius KappaCCD diffractometer. Structure solution was performed using SHELXS-86 [G. M. Sheldrick, SHELXS-86, Computer Program for Crystal Structure Determination, University of Göttingen, Germany, 1986], and refinement was by full-matrix least squares on F^2 using SHELXL-97 [G. M. Sheldrick, SHELXL-97, Computer Program for Crystal Structure Refinement, University of Göttingen, Germany, 1997] software.

In *R,R*-**1** the asymmetric unit consists of two boronic acid molecules plus half a molecule of dichloromethane positionally disordered over two sites in a 35:15 ratio. The boronic acid molecules are associated via hydrogen bonding between the acidic hydrogen atoms (H1−H8) of the boronic acid functionalities and the lone pairs on the tertiary amine nitrogen atoms. H1−H8 were included at calculated positions in the refinement, but their isotropic atomic displacement parameters were refined without restraint.

In the *S,S*-**1** with L-tartaric acid complex the asymmetric unit contains one molecule of the boronate, plus two fragments of methanol, each with 50% occupancy. Despite collecting four independent data sets for this structure, on substantially sized, good quality crystals, a relatively premature fall off in diffraction intensity was unavoidable. On refining the structural model to the stage where the disordered methanol presented herein was identified, it became obvious that there was additional partial occupancy (range 20−40%) solvent fragments located in the lattice. It was also evident from early in the refinement procedure that there is apparent void space in the lattice (confirmed by PLATON). Thus, a data squeeze was implemented, and the refinement presented here takes account of this. (R_1 prior to this adjustment had converged to 11.42%).

Synthesis. *S,S*-(+)-Bisboronic Acid (1). 9,10-Anthracenedicarboxaldehyde (2.00 g, 6.80 mmol) and (*S*)-(−)-α-methylbenzylamine (3.00 g, 24.8 mmol) were dissolved in 90 mL EtOH/THF (7:2) and a few drops of acetic acid were added as catalyst. The mixture was heated with stirring at reflux for 6 h under an N_2 atmosphere. The mixture was then cooled to room temperature and filtered under vacuum to give *S,S*-**4** (2.30 g, 76.8%) as yellow needles. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.44 (s, 2H), 8.40 (m, 4H), 7.63−7.33 (m, 14H), 4.90 (q, 2H, J = 6.0 Hz), 1.87 (d, 6H, J = 6.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.4, 145.2, 131.2, 129.8, 129.1, 127.6, 127.3, 126.7, 125.7, 72.2, 25.7. *S,S*-**4** was used without further purification.

S,S-**4** (1.13 g, 2.56 mmol) was dissolved in dry THF, and then $NaBH_4$ (0.96 g, 25.4 mmol) was added and the mixture stirred at room temperature until the reduction was finished (the reaction was monitored by TLC using alumina plates with dichloromethane as eluent). The solvent was removed and the residue dissolved in dichloromethane then washed with brine. The organic phase was dried over anhydrous $MgSO_4$ and the solvent removed under vacuum to give *S,S*-**5** (0.900 g, 78.9%) as a light-yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 (m, 4H), 7.55−7.27 (m, 14H), 4.44 (d, 2H, J = 12.0 Hz), 4.40 (d, 2H, J = 12.0 Hz), 4.08 (q, 2H, J = 6.0 Hz), 1.41 (d, 6H, J = 6.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 146.1, 132.5, 130.5, 129.0, 127.6, 127.4, 125.9, 125.3, 59.7. 44.6, 25.1. *S,S*-**5** was used without further purification.

(26) Franzen, S.; Ni, W.; Wang, B. *J. Phys. Chem. B* **2003**, *107*, 12942.

S,S-**5** (0.95 g, 2.14 mmol) and 2-(2-bromomethyl)-1,3,2-dioxaborinane (1.75 g, 6.86 mmol) were dissolved in 30 mL of dry acetonitrile, and then K_2CO_3 (1.18 g, 8.54 mmol) was added and the mixture heated under reflux with stirring for 10 h. The solvent was evaporated and water added to the residue, the resultant heterogeneous mixture was then extracted with dichloromethane. The organic phases were recombined and dried over $MgSO_4$ and the solvent removed to give a dark-yellow solid. The solid was dissolved in acetone and water was added dropwise with stirring until a light-yellow precipitate formed. The solid was vacuum filtered and washed with an acetone/water mixture to give *S,S*-**1 (**1.20 g, 78.5%) as a light-yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$, and a few drops of CD_3OD) δ 7.75−7.09 (m, 26H), 4.56 (d, 2H, J = 15.0 Hz), 4.41 (d, 2H, J = 12.0 Hz), 3.95 (q, 2H, J = 6.0 Hz), 3.67 (s, 4H), 1.59 (d, 6H, J = 6.0 Hz); *m*/*z* (FAB) 1252.0 ([M + 4NOBA − $4H_2O$]$^+$, 7%). Anal. Calcd for $C_{46}H_{46}B_2N_2O_4 \cdot 0.5H_2O$: C, 76.6; H, 6.57; N, 3.88. Found: C, 76.5, H, 6.49; N, 3.78. $[\alpha]_D^{22}$ = +28 ± 1° (c = 1.0, CH_3OH).

***R,R*-(−)-Bisboronic Acid (1).** 9,10-Anthracenedicarboxaldehyde (2.00 g, 6.80 mmol) and (*R*)-(+)-α-methylbenzylamine (3.00 g, 24.8 mmol) were dissolved in 90 mL of EtOH/THF (7:2) and a few drops of acetic acid were added as catalyst. The mixture was heated under reflux with stirring for 6 h under an N_2 atmosphere. The mixture was then cooled to room temperature and filtered under vacuum to give *R,R*-**4** (2.30 g, 76.8%) as yellow needles. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.37 (s, 2H), 8.26 (m, 4H), 7.52−7.14 (m, 14H), 4.79 (q, 2H, J = 6.0 Hz), 1.74 (d, 6H, J = 6.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.5, 145.1, 131.2, 129.8, 129.1, 127.6, 127.2, 126.7, 125.7, 72.2, 25.6. *R,R*-**4** was used without further purification.

R,R-**4** (1.57 g, 3.56 mmol) was dissolved in 40 mL of dry THF, and then $NaBH_4$ (1.70 g, 44.9 mmol) was added and the mixture stirred at room temperature until the reduction was finished (the reaction was monitored using TLC alumina plates with dichloromethane as eluent). The solvent was removed and the residue dissolved in dichloromethane and washed with brine. The organic phase was dried over anhydrous $MgSO_4$ and the solvent removed under vacuum, to give *R,R*-**5** (0.950 g, 60.1%) as a light-yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07 (m, 4H), 7.47−7.27 (m, 14H), 4.44 (d, 2H, J = 12.0 Hz), 4.35 (d, 2H, J = 12.0 Hz), 3.99 (q, 2H, J = 6.0 Hz), 1.68 (b, 2H); 1.32 (d, 6H, J = 6.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 146.1, 132.5, 130.5, 128.9, 127.7, 127.4, 125.9, 125.3, 59.7, 44.6, 25.1. *R,R*-**5** was used without further purification.

R,R-**5** (0.95 g, 2.14 mmol) and 2-(2-bromomethyl)-1,3,2-dioxaborinane (1.75 g, 6.86 mmol) were dissolved in 30 mL of dry acetonitrile, and then K_2CO_3 (1.18 g, 8.54 mmol) was added and the mixture heated under reflux with stirring for 10 h. The solvent was evaporated and water added to the residue, the resultant heterogeneous mixture was then extracted with dichloromethane. The organic phases were recombined and dried over $MgSO_4$ and the solvent was removed. The solid was then dissolved in acetone and water was added dropwise with stirring until a light-yellow precipitate formed. The solid was vacuum filtered and washed with an acetone/water mixture to give *R,R*-**1** (1.01 g, 66.4%) as a light-yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$ and a few drops of CD_3OD) δ 7.72−7.06 (m, 26H), 4.55 (d, 2H, J = 12.0 Hz), 4.39 (d, 2H, J = 15.0 Hz), 3.94 (q, 2H, J = 6.0 Hz), 3.78 (s, 4H), 1.58 (d, 6H, J = 6.0 Hz); *m*/*z* (FAB) 1252.1 ([M + 4NOBA − $4H_2O$]$^+$, 7%). Anal. Calcd for $C_{46}H_{46}B_2N_2O_4 \cdot H_2O$: C, 75.6; H, 6.62; N, 3.83. Found: C, 75.3, H, 6.54; N, 3.74. $[\alpha]^{22}_D$ = −27 ± 1° (c = 1.0, CH_3OH).

***S*-(+)-Monoboronic Acid (2).** 9-Anthracenecarboxaldehyde (2.00 g, 9.70 mmol) and (*S*)-(−)-α-methylbenzylamine (1.76 g, 14.5 mmol) were dissolved in 50 mL of EtOH/THF (7:2) and few drops of acetic acid were added as catalyst. The mixture was heated under reflux with stirring for 6 h under an N_2 atmosphere. The mixture was then cooled to room temperature and filtered under vacuum to give *S*-**6** (2.70 g, 90.0%) as yellow needles. ^{1}H NMR (300 M Hz, $CDCl_3$) δ 9.44 (s, 1H), 8.40 (m, 3H), 7.94 (m, 2H), 7.17−7.53 (m, 9H), 4.79 (q, 1H, J = 9.0 Hz), 1.76 (d, 3H, J = 9.0 Hz); ^{13}C NMR (75 M Hz, $CDCl_3$) δ 159.3, 145.3, 131.7, 130.4, 129.6, 129.2, 129.1, 129.0, 128.7, 127.5, 127.2, 127.0, 126.3, 125.6, 125.2, 72.2, 25.8. *S*-**6** was used without further purification.

S-**6** (1.90 g, 6.14 mmol) was dissolved in 50 mL of dry THF, and then $NaBH_4$ (1.13 g, 30.0 mmol) was added and the mixture was stirred at room temperature until the reduction was finished. The solvent was removed and the residue dissolved in dichloromethane and washed with brine. The organic phase was dried over anhydrous $MgSO_4$ and the solvent removed under vacuum to give *S*-**7** (1.55 g, 81.1%) as a light yellow oil which slowly solidified at room temperature. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.22 (s, 1H), δ 8.00 (m, 2H), δ 7.84 (m, 2H), 7.43−7.19 (m, 9H), 4.40 (d, 1H, J = 12.0 Hz), 4.32 (d, 1H, J = 12.0 Hz), 3.94 (q, 1H, J = 6.0 Hz), 1.41 (d, 3H, J = 6.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 146.2, 132.4, 132.1, 130.7, 129.6, 129.1, 127.7, 127.6, 127.5, 126.4, 125.1, 124.7, 59.8, 44.6, 25.1. *S*-**7** was used without further purification.

S-**7** (1.30 g, 4.17 mmol) and 2-(2-bromomethyl)-1,3,2-dioxaborinane (1.17 g, 4.59 mmol) were dissolved in 50 mL of dry acetonitrile, and then K_2CO_3 (0.63 g, 4.56 mmol) was added and the mixture was stirred under reflux overnight. The solvent was evaporated and water added to the residue, the resultant heterogeneous mixture was then extracted with dichloromethane. The organic phases were recombined and dried over anhydrous $MgSO_4$ and the solvent was removed. The solid was then dissolved in acetone and water was added dropwise with stirring until a light-yellow precipitate formed. The solid was vacuum filtered and washed with an acetone/water mixture to give *S*-**1** (96 mg, 5.2%) as a light yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.31 (s, 1H), 7.85 (d, 2H, J = 9.0 Hz), 7.72 (d, 2H, J = 6.0 Hz), 7.39−7.17 (m, 14H), 4.55 (d, 1H, J = 12.0 Hz), 4.49 (d, 1H, J = 18.0 Hz), 3.94 (q, 1H, J = 6.0 Hz), 3.87 (d, 1H, J = 15.0 Hz), 3.94 (d, 1H, J = 12.0 Hz), 1.64 (d, 3H, J = 6.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 142.0, 141.2, 136.9, 132.2, 131.7, 131.5, 130.7, 130.0, 129.6, 128.9, 128.3, 127.7, 126.3, 125.1, 124.7, 59.4, 57.3, 44.4, 31.3; *m*/*z* (FAB) 716.2 ([M + 2NOBA − $2H_2O$]$^+$, 30%). Anal. Calcd for $C_{30}H_{28}BNO_2$: C, 80.9; H, 6.34; N, 3.15. Found: C, 80.5, H, 6.31; N, 3.12. $[\alpha]^{22}_D$ = +56 ± 1° (c = 1.0, CH_3OH).

***R*-(−)-Monoboronic Acid (2).** 9-Anthracenecarboxaldehyde (2.00 g, 9.70 mmol) and (*S*)-(−)-α-methylbenzylamine (1.76 g, 14.5 mmol) were dissolved in 50 mL of EtOH/THF (7:2) and a few drops of acetic acid were added as catalyst. The mixture was heated under reflux with stirring for 6 h under an N_2 atmosphere. The mixture was then cooled to room temperature and filtered under vacuum to give *R*-**6** (2.00 g, 66.6%) as yellow needles. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.35 (s, 1H), 8.31 (m, 3H), 7.94 (m, 2H), 7.17−7.49 (m, 9H), 4.72 (q, 1H, J = 6.0 Hz), 1.69 (d, 3H, J = 6.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.3, 145.4, 131.7, 130.5, 129.2, 129.1, 127.5, 127.3, 127.1, 125.7, 125.3, 72.3, 25.8. *R*-**6** was used without further purification.

R-**6** (1.90 g, 6.14 mmol) was dissolved in 50 mL of dry THF, and then $NaBH_4$ (1.13 g, 30.0 mmol) was added and the mixture stirred at room temperature until the reduction was finished. The solvent was removed and the residue dissolved in dichloromethane and washed with brine. The organic phase was dried over anhydrous $MgSO_4$ and the solvent removed under vacuum to give *R*-**7** (1.63 g, 85.2%) as a light yellow oil which slowly solidified at room temperature. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.23 (s, 1H), δ 8.01 (m, 2H), 7.83 (m, 2H), 7.43−7.21 (m, 9H), 4.40 (d, 1H, J = 12.0 Hz), 4.32 (d, 1H, J = 12.0 Hz), 3.94 (q, 1H, J = 6.0 Hz), 1.29 (d, 3H, J = 6.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 146.2, 132.4, 132.1, 130.7, 129.6, 129.1, 127.7, 127.6, 127.4, 126.4, 125.4, 124.7, 59.8, 44.6, 25.1. *R*-**7** was used without further purification.

R-**7** (1.30 g, 4.17 mmol) and 2-(2-bromomethyl)-1,3,2-dioxaborinane (1.17 g, 4.59 mmol) were dissolved in 50 mL of dry acetonitrile, and then K_2CO_3 (0.63 g, 4.56 mmol) was added and the mixture heated under reflux with stirring overnight. The solvent was evaporated and water added to the residue, the resultant heterogeneous mixture was

then extracted with dichloromethane. The organic phases were combined, dried over anhydrous $MgSO_4$ and the solvent removed. The solid was then dissolved in acetone and water was added dropwise with stirring until a light-yellow precipitate formed. The solid was vacuum filtered and washed with an acetone/water mixture to give *R*-**1** (0.260 g, 14.0%) as a light yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.31 (s, 1H), 7.86 (d, 2H, J = 6.0 Hz), 7.72 (d, 2H, J = 6.0 Hz), 7.40−7.17 (m, 14H), 4.55 (d, 1H, J = 15.0 Hz), 4.49 (d, 1H, J = 15.0 Hz), 4.04 (q, 1H, J = 6.0 Hz), 3.88 (d, 1H, J = 12.0 Hz), 3.77 (d, 1H, J = 12.0 Hz), 1.64 (d, 3H, J = 9.0 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 141.9, 141.2, 136.9, 132.2, 131.7, 131.5, 130.7, 130.0, 129.6, 128.9, 128.3, 127.7, 126.3, 125.2, 124.7, 59.4, 57.3, 44.4, 31.3; *m*/*z* (FAB) 715.9 ([M + 2NOBA − $2H_2O$]$^+$, 30%). Anal. Calcd for $C_{30}H_{28}BNO_2$: C, 80.9; H, 6.34; N, 3.15. Found: C, 80.6, H, 6.23; N, 3.18. $[\alpha]^{22}_D = -57 \pm 1°$ (c = 1.0, CH_3OH).

Conclusions

In conclusion the chiral fluorescent sensor **1** was found to be highly sensitive, chemoselective, and enantioselective to sugar acids, such as tartaric acid, glucaric acid, and gluconic acid. The enantioselectivity is as high as 550:1 and the detection limit for some sugar acids is in the micromolar range. Sensor **1** with high sensitivity, chemoselectivity, and enantioselectivity has clearly demonstrated the value of the modular approach to the construction of chiral sensors. These results, together with the unambiguous structural data we report, should aid further development and application of enantioselective chemical sensors.

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Supporting Information Available: Original titration curves for the entries in Table 1 and X-ray crystallographic data (PDF, CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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