



Combining ferrocene, thiophene and a boronic acid: a hybrid ligand for reagentless electrochemical sensing of *cis*-diols



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ABSTRACT

A redox-active affinity ligand suitable for reagentless sensing of *cis*-diols was synthesised and characterised. 4-[(Ferrocenylamino)methyl]thiophene-3-boronic acid (FcTBA) was allowed to interact with the model *cis*-diol, sorbitol. A discrete, cathodic shift of the redox potential was observed upon interaction of FcTBA with sorbitol thus providing simultaneous differentiation between the free and bound forms of this sensor molecule. Similar behaviour was observed also for FcTBA co-immobilised with thiophene in a mixed self-assembled monolayer on a gold electrode.

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The idea of reagentless sensors still remains in the focus of analytical chemists.^{1,2} This approach enables easy determination of the target analyte without any necessary additions of reagents, and only a specifically interacting and directly responding probe is used for this purpose. In particular, electrochemical transducers are very suitable for such applications as they can be easily miniaturised, and samples can be examined without extensive purification. Although several attempts to prepare such types of (bio)sensors were realised with enzymes,^{2–5} the most suitable route utilises an affinity interaction, for example, a specific receptor molecule is immobilised directly on the sensing surface. One such example is the artificial receptor for saccharides based on the complexation abilities of boronic acid.¹

Boronic acid (BA) has been widely employed for homogeneous sensing applications as it forms reversible cyclic esters with *cis*-diols. Although the majority of these sensors rely on optically active derivatives, electrochemical systems were also studied.^{1,6,7} It is surprising that electrochemically responsive systems are much less pursued since some of them exhibit very interesting ability to discriminate between free and bound forms of BA in a homogeneous assay.^{1,8} Moreover, a simple sensor for *cis*-diols can be ready to use after linking of the receptor molecule to the electrode surface. Such electrochemical heterogeneous sensors based on boronic acid employ either direct or indirect sensing modes. The

indirect modes are based on non-electroactive derivatives of boronic acid immobilised on the electrode. The presence and subsequent binding of the analyte to the modified electrode result in a variation of a particular physical property which is followed by suitable electrochemical methods. This includes the permeability of the attached layer of BA-*cis*-diol, which is probed by a suitable electroactive label⁹ or through accumulation of charge due to binding of the charged species.^{10,11} A more interesting and simpler sensing platform provides direct sensing in which molecules exhibit simultaneously specific binding, electroactivity and simple anchorage to the electrode surface. This provides a real electrochemical reagentless sensor in which the signalling event stems from the change of the inherent chemico-physical property of the surface confined sensor molecule.

The sensing ability of such molecular ligands, either in the homogeneous or heterogeneous phase, is given by the change of the redox potential upon binding of an analyte (valid also for electrochemically active BAs generally). Depending on the experimental conditions (especially scan rate of potential), two possible modes of behaviour exist; firstly, there can be a ‘continuous shift’ of redox potential upon the sensing event.^{12,13} Secondly, a ‘discrete shift’ of the electrochemical potential is observed where two individual peaks corresponding to the free and bound forms are evident.^{8,14–19} Furthermore, the shift of the electrochemical potential can be either anodic¹⁵ or cathodic^{8,14,16–20} depending on the structure of the sensor molecule. A cathodic shift was observed for molecules where the binding event was conveyed

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directly from the boron atom. The opposite effect was observed for a molecule where the interaction with the *cis*-diol was mediated by a nitrogen atom in close proximity to the signalling unit.

Basically, the previously mentioned elegant platform of a boronic acid based reagentless-like sensor has only been realised by two approaches. Murakami et al. introduced a sensor working in aqueous solutions consisting of phenylboronic acid, 4,4'-bipyridyl and an alkylthiol.²⁰ The published results were surprising as the boronic acid residue was directly linked to the electroactive centre and a shift of potential of less than 20 mV was observed in the presence of rather high concentrations of the diol. Moreover no discrete peaks due to the free and bound forms were visible and even the presence of a compound with no binding sites for boronic acid influenced the electrochemical potential. Nicolas et al. have prepared different types of redox active polymers with a pendant boronic acid.^{17–19} These were electropolymerised on the electrode surface and the modified electrode was used for monitoring of fluoride anions. Although separate peaks corresponding to the free and bound forms of boronic acid seemed promising for further sensing applications, unfortunately the polymer only exhibited stable electroactive properties in an organic solvent such as acetonitrile.

Several redox-active compounds such as Fe(II)-bipyridine,¹⁸ phenazine,²¹ tetrathiafulvalene²² and ferrocene^{8,12–16,23} were tested as signalling residues in the homogeneous phase. Nevertheless, ferrocene seems to be the most promising for broader analytical applications as its derivatives are small and water soluble molecules. As the electrochemical properties of all the cited boronic acids containing ferrocene were studied only in solution, an obvious further step is modification of the molecule aimed at direct linkage to the electrode surface.

All the previously expressed aspects were considered in the design and synthesis of the novel molecule, 4-[(ferrocenylamino)methyl]thiophene-3-boronic acid (FcTBA). FcTBA includes all the modules necessary for such a reagentless receptor: a boronic acid, ferrocene and thiophene as interacting, signalling parts and the unit enabling simple anchorage to the surface, respectively. The sugar alcohol, sorbitol with known high affinity for boronic acids^{8,14,16} was chosen as a model *cis*-diol representing the structural motif typical for many biologically relevant compounds such as saccharides.

FcTBA **4** was synthesised according to Scheme 1. Aminoferrocene (**1**) (10.05 mg, 50 mmol) and 4-formylthiophene-3-boronic acid (**2**) (31.19 mg, 200 mmol) were dissolved in 50% ethanol in water solution (50 ml) and stirred. After several minutes, initial precipitation of the red Schiff base adduct **3** occurred due to the polarity of the solvent. The solution was further stirred for 2 h at room temperature to complete the reaction. The solvent was removed and the residue was washed with distilled water and dried at 70 °C (yield 69%). The dried product (11.65 mg, 34 mmol) was dissolved in ethanol (5.74 ml) and NaBH₄ (5.74 mg, 152 mmol) was added. After 15 min of stirring, the solvent was removed under reduced pressure. The resulting reddish brown solid was washed thoroughly with water and dried at 70 °C (yield 93%). The structure of intermediate **3** and the final

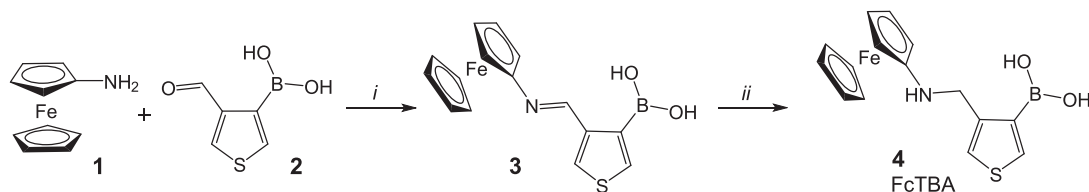
product, FcTBA **4** was confirmed by mass spectrometry (Figs. S1–S4) and NMR spectroscopy (Fig. S5).

The behaviour of both **3** and **4** in an aqueous solution in the presence of sorbitol was studied by cyclic voltammetry (CV).²⁴ The experiment with intermediate **3** provided results comparable with the literature.²⁵ The minor decomposition of the original non-reduced imine form of FcTBA **3** over time was enhanced upon interaction with sorbitol. The progress of the reaction was monitored by the decrease of the signal of **3** (region around 475 mV) and the increase of the signal due to free aminoferrocene (20 mV) (Fig. 1).

The response of the compound of interest, FcTBA to the addition of sorbitol (Fig. 2) was compared with the behaviour of ferroceneboronic acid (FcBA) under the same conditions (Fig. S7). The expected discrete shift of the redox potential was observed upon addition of the *cis*-diol in both cases. Only the response of the high affinity oxidised form of the electrochemically active boronic acid^{8,14} was used for further evaluation of its interaction with *cis*-diols. The observed equilibrium association constants, K_a' , of the oxidised forms of both BAs with sorbitol were thus determined from the reductive peak currents in CV measurements. The values of K_a' for FcTBA and FcBA were calculated according to the modified previously published method²⁶ as 3400 and 6200 M^{−1}, respectively.²⁴ Only data for concentrations up to 2 mM of sorbitol were considered.

Intramolecular binding between atoms of nitrogen and boron in the structure of sensory BAs is a useful molecular motif nowadays accepted as a standard.^{27–29} This interaction facilitates transformation of boronic acid into its tetrahedral form as required for strong binding, not only in aqueous solutions.³⁰ Although this constitutional structural element is found in the molecule being studied, the existence of this interaction was questioned for similar compounds in the literature,³⁰ and by our electrochemical results as well. The determined association constants for both BAs under particular conditions seem to exclude a strong B–N bond in FcTBA. Nevertheless, a careful comparison of the data presented in Figure 2 and Figure S7 gives two visible differences. Firstly, the magnitude of the redox potential shifts after the binding of sorbitol to different signalling molecule. The difference between the free and bound forms of oxidised FcTBA and those of FcBA is 100 mV and 225 mV, respectively. Secondly, the oxidised form of FcTBA with a concentration of 4 mM sorbitol (and higher) becomes saturated, while there is still some unbound form in the case of FcBA. Furthermore, NMR spectroscopy revealed changes in the close environment of the nitrogen atom after binding of sorbitol in DMSO (Figs. S5 and S6). These findings do not definitely rule out certain intramolecular interactions and indicate high dependency of the electrochemical response on the structure of BA. Further investigation of these effects is presently underway.

As the FcTBA molecule was characterised electrochemically in the homogeneous phase, the heterogeneous assay was also investigated. This molecule has a thiophene residue spontaneously creating self-assembled monolayers on gold.³¹ Thus the gold electrode was modified simply by overnight incubation in a mixture consisting of FcTBA and thiophene (2 and 1 mM, respectively, in ethanol).



Scheme 1. Synthesis of FcTBA (**4**): Reagents and conditions: (i) EtOH/H₂O, rt, 69%, (ii) EtOH, NaBH₄, rt, 93%.

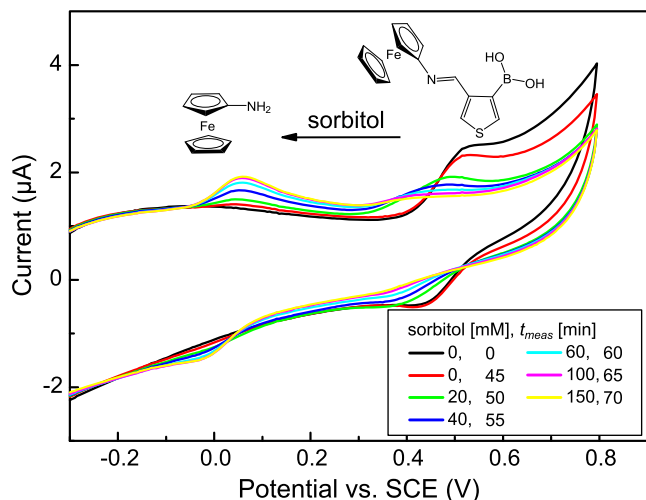


Figure 1. The decomposition of **3** upon interaction with sorbitol in DMSO/phosphate buffer, pH 7.4 (3:1) as studied by cyclic voltammetry with a glassy carbon disc electrode; t_{meas} represents the time at which the CV measurement was performed; only electrochemically active compounds are depicted.

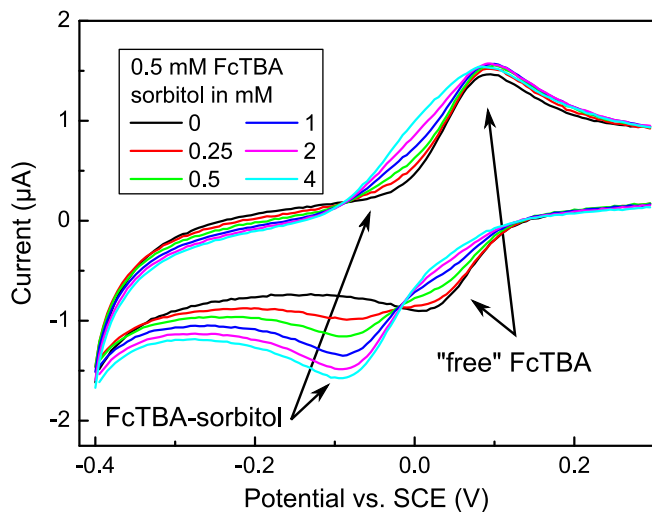


Figure 2. The interaction of FcTBA with sorbitol in DMSO/phosphate buffer, pH 7.4 (3:1) studied by cyclic voltammetry with a glassy carbon disc electrode.

When the modified electrode was transferred to the pure electrolyte, the cyclic voltammogram revealed the redox pair of a ferrocene moiety. A similar response as in the case of the homogeneous assay upon the addition of sorbitol was observed for the FcTBA-modified electrode—two discrete reductive peaks corresponding to the free and bound forms of electrochemically active BA (Fig. 3, for dynamic behaviour of the interaction, see Fig. S8). The only exception was a lowered affinity to sorbitol. This is probably due to the fact that the molecule of FcTBA is in a rigid environment of stacked thiophene moieties and consequently its mobility and rotation abilities are thus limited as well as the diffusion of sorbitol. As the BA-*cis*-diol interaction is not stable at lower pH values, the sensor was incubated for 1 min in 0.1 M HCl. This resulted in regeneration of the sensing surface as only the peak corresponding to the free form was observed in the pure electrolyte (Fig. 3). Minor dissociation of FcTBA from the electrode surface can be observed during the course of the measurements.

In conclusion, the concept of a surface-confined affinity receptor was validated by the synthesis of FcTBA combining signalling

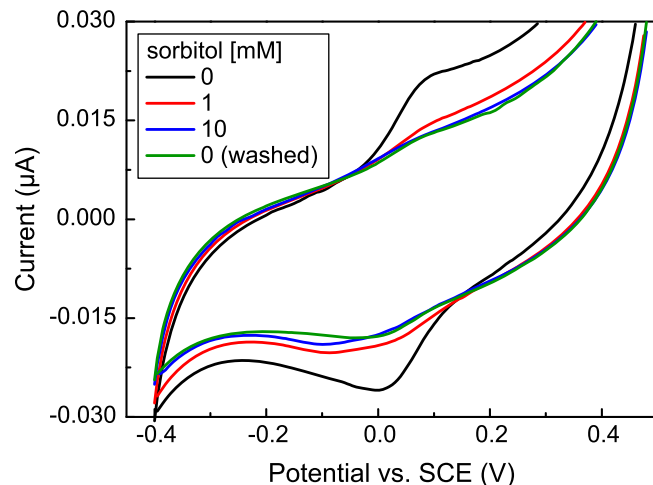


Figure 3. Electrochemical behaviour of FcTBA immobilised on a gold electrode in the absence and in the presence of sorbitol; experimental conditions are the same as in Figure 2 with the exception of a gold disc working electrode.

(ferrocene), recognition (boronic acid) and anchorage (thiophene) modules. This represents a promising analytical approach enabling reagentless detection of biologically active compounds. The inherent ability of electrochemically active boronic acids to discriminate between their free and bound forms allows the design of original sensing applications. The sensing is based on two discrete peaks appearing in the voltammogram after binding of the *cis*-diol and only the ratio of peak heights is required for obtaining the desired information. Due to this ratiometric approach, where one of the peaks serves as an internal reference, the potentially disturbing matrix effects become eliminated. It should be mentioned that this effect is employed only within 0 and 100% occupancy of the available binding sites. The alternative techniques relying on the simple shift of the electrochemical potential of the redox active compound must have accurately defined its initial value in the given working environment or sample matrix. Moreover, this concept can be extended for the practical sensing of other molecules lacking redox active groups.

In the near future the close environment of the boronic acid binding site and the anchoring unit will be further investigated and modified in order to obtain derivatives with finely tuned *cis*-diol affinities, and means of immobilization suitable for different sensing surfaces.

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Supplementary data

Supplementary data (supplementary material includes mass spectrometry, NMR binding studies, electrochemical experiments and determination of binding constants) associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.tetlet.2014.04.036>. These data include MOL files and InChIKeys of the most important compounds described in this article.

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