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A modular electrochemical sensor for saccharides†

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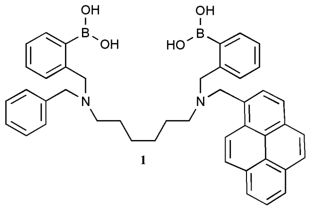
Received (in Cambridge, UK) 5th August 2002, Accepted 2nd September 2002
First published as an Advance Article on the web 18th September 2002

A modular electrochemical saccharide sensor using ferrocene has been prepared which contains two boronic acid receptor groups and hexamethylene linker.

Much recent attention has been paid to the development of synthetic molecular receptors with the ability to recognise neutral organic species, including saccharides.[1–3] Boronic acid receptors for saccharides have attracted considerable interest.[2,3] Boronic acids are known to bind saccharides *via* covalent interactions in aqueous basic media. The most common interaction is with *cis*-1,2- or 1,3-diols of saccharides to form five- or six-membered rings respectively. The interaction of a boronic acid (Lewis acid) and neighbouring tertiary amine (Lewis base) is strengthened on saccharide binding.[2,3]



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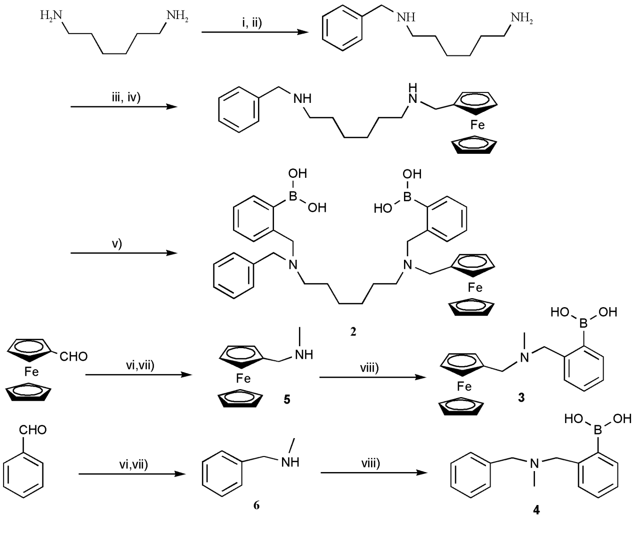
Over the last few years we have been interested in developing new sensors selective for saccharides employing a modular approach. The basic idea was to break a sensor into three components; receptor units, linker units, and 'read-out' units. The approach can be illustrated by describing the D-glucose selective fluorescence sensor **1** which contains two boronic acid units (receptors), hexamethylene unit (D-glucose selective linker), and pyrene unit (fluorophore-'read-out').[4,5] Using compound **1** as a model any new saccharide selective sensor requires at least two boronic acid units, one linker unit, and a 'read-out' unit. Two or more boronic acid units as with sensor **1** are required because only through two points binding can saccharide selectivity be controlled. One 'read-out' unit is required as with sensor **1** to report on saccharide binding. Also a linker is required and the choice of linker is very important since it will determine the selectivity of the sensor. With compound **1**, a hexamethylene linker results in D-glucose selectivity.[4,5] Following the criteria given above and the requirements for electrochemical detection,[6] we designed the electrochemical saccharide sensor **2** with two boronic acid units (saccharide selectivity), one ferrocene unit and a hexamethylene linker unit (D-glucose selectivity).

Electrochemical detection of saccharides by enzymatic decomposition of saccharides is well known.[7] The development of boronic acid based electro-active saccharide receptors could provide selectivity for a range of saccharides. Chiral ferrocene-boronic acid derivatives have been synthesized and tested for chiral electrochemical detection of monosaccharides.[8] A recent paper by Moore and Wayner has explored the redox switching of carbohydrate binding with commercial ferrocene boronic acid.[9] With both of the above systems, D-fructose selectivity was observed which is the inherent selectivity for all monoboronic acids.[10] Also, for these two systems the observed binding with D-glucose is very weak, the calculated stability constants are < 20 dm^3 mol^{-1}.

With this research we set out to design a diboronic acid system which could be used to control saccharide selectivity through two-point binding. Synthesis of **2** and reference compounds **3**,[11] **4** and **5** was achieved according to Scheme 1 from readily available starting materials.‡

Differential pulse voltammograms (DPV) were recorded for reference compound **5** (5.0×10^{-5} mol dm^{-3}),§ a 1:1 mixture of compound **3** + **4** (5.0×10^{-5} mol dm^{-3})§ and **2** (5.0×10^{-5} mol dm^{-3}) (Fig. 1) with D-glucose (0–0.1 mol dm^{-3}) in aqueous methanolic buffer solution [52.1 wt% methanol at pH 8.21 (KCl, 0.01000 mol dm^{-3}; KH_2PO_4, 0.002752 mol dm^{-3}; Na_2HPO_4, 0.002757 mol dm^{-3})]. DPV removes the effect of electrode capacitive charging, resulting in measurement of only the Faradaic proceeses and hence much higher signals than conventional voltammetries.[12] The DPV were recorded with an Ecochemie μ-Autolab potentiostat, using a single-compartment cell fitted with a glassy carbon electrode for working electrode (0.28 cm^2), a platinum plate for counter electrode, and a Ag/AgCl reference electrode. From Fig. 1 it can be seen that the oxidation potential of the ferrocene becomes more anodic on binding with saccharides. The interaction of the boronic acid and neighbouring amine is strengthened on saccharide binding,[2,3] thereby, reducing the electron density on the neighbouring amine. This in turn destabilises the ferrocenium ion at higher concentrations of saccharides, resulting in a more anodic ferrocene oxidation overpotential (Scheme 2).



Scheme 1 Synthesis of **2**, **3**, **4** and **5**. *Reagents* (yields): i) benzaldehyde, *p*-toluenesulfonic acid, THF/EtOH: ii) $NaBH_4$, 79% (2 steps): iii) ferrocene-calboxaldehyde, THF/MeOH: iv) $NaBH_4$, 76% (2 steps): v) 2-(2-bromobenzyl)-1,3-dioxaborinane, K_2CO_3, MeCN 69%: vi) methylamine, THF/MeOH: vii) $NaBH_4$, 74% (**5**), 55% (**6**) (2 steps): viii) 2-(2-bromobenzyl)-1,3-dioxaborinane, K_2CO_3, MeCN 42% (**3**), 10% (**4**).

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† Electronic supplementary information (ESI) available: observed DPV for **3** + **4** and **5**. See http://www.rsc.org/suppdata/cc/b2/b207643h/

DOI: 10.1039/b207643h

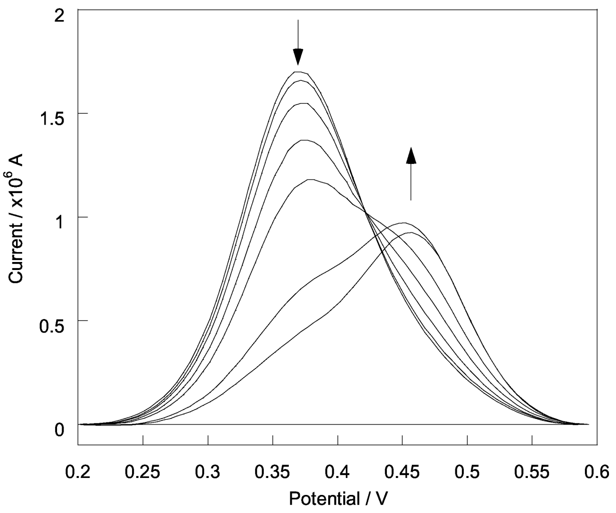
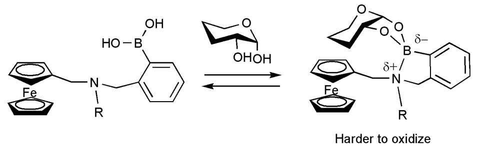


Fig. 1 Differential pulse voltammograms of **2** (5.0×10^{-5} mol dm^{-3}) with different concentration of D-glucose (0, 1.11×10^{-4}, 3.89×10^{-4} 6.66×10^{-4} 1.04×10^{-3}, 1.03×10^{-2}, 1.02×10^{-1} mol dm^{-3}) at pH 8.21 in 52.1 wt% methanol. The area of the glassy carbon electrode is 0.28 cm^2. Voltammetric parameters are as follows: scan rate, 20 mV s^{-1}; modulation time, 50 ms; interval time, 500 ms; step potential, 5.1 mV; modulation amplitude 25.05 mV. The lack of an isobestic point is attributed to viscosity effects.‡



Scheme 2

The stability constants (K) of electrochemical sensors **2** and the 1 : 1 mixture of **3** + **4** were calculated by fitting the current intensity at 0.357 V *vs.* saccharide concentration and are given in Table 1.[13,14]

The relative stability constants of the diboronic acid **2** relative to the 1 : 1 mixture of monoboronic acids **3** + **4** are given in Table 1. A 1 : 1 mixture of monoboronic acids **3** + **4** was used as the reference to alow direct comparison with the diboronic acid system **2**. Using a 1 : 1 mixture ensures that the same concentration of both ferrocene and boronic acid groups are present in both cases.

Although the highest binding constant for compound **2** is with D-fructose, cooperative binding of the two boronic acid groups is clearly observed as illustrated by the stability constant differences between the mono- and di-boronic acid compounds (sensors **2** and **3** + **4**, respectively). In particular the stability constant K of diboronic acid sensor **2** with D-glucose was 40 times greater than with monoboronic sensor **3 + 4**, whereas the stability constant K of diboronic acid sensor **2** with D-fructose was only 4 times stronger than monoboronic sensor **3 + 4.** These results are not surprising since it is well known that D-glucose easily forms 1 : 1 cyclic complexes with di-boronic acids, whereas D-fructose tends to form 2 : 1 acyclic complexes with di-boronic acids.[2,3]

Table 1 Stability constant K (determination of coefficient; r^2) for the saccharide complexes of molecular sensors **2** and **3 + 4**

	K/mol^{-1} dm^3 at 25 °C		
Saccharide	**2**	**3+4**	**2/3 + 4**
D-Glucose	684 ± 54 (0.99)	17 ± 2 (0.99)	40
D-Fructose	1478 ± 72 (1.00)	362 ± 5 (1.00)	4
D-Galactose	782 ± 72 (0.99)	47 ± 2 (1.00)	17
D-Mannose	149 ± 9 (1.00)	54 ± 8 (0.99)	3

In conclusion, we have shown that it is possible to prepare an electrochemical sensor **2** with enhanced D-glucose (40 fold) and D-galactose (17 fold) binding employing simple building blocks and using a modular approach. We believe that these results could be applied in the development of new saccharide selective electrochemical sensors. Our ongoing research in directed towards new modular electrochemical sensors with different linkers and electroactive units.

T. D. J. wishes to acknowledge the Royal Society, the EPSRC, and Beckman-Coulter for support. S. A. wishes to acknowledge Beckman Coulter for support through the award of a Postdoctoral Fellowship. S. U. wishes to acknowledge the EPSRC for support through the award of an EPSRC studentship. A. T. A. J. wishes to acknowledge the Nuffield foundation for an equipment grant. We wish to thank Dr Noel W. Duffy for helpful advice and discussions. We would also like to acknowledge the support of the University of Bath.

Notes and references

‡ *Selected data* for **2**: mp 155–158 ° C (decomp.); *m*/*z* (FAB) 1212 ([M + H + 4(3-$HOCH_2C_6H_4NO_2$) − $4H_2O$]+, 95%); Found: C, 69.25; H, 7.07; N, 4.12. $C_{38}H_{46}B_2FeN_2O_4$-H_2O+0.05 $CHCl_3$ requires C, 69.21; H, 6.74; N, 4.24%. δ_H(300 MHz, $CDCl_3$ + CD_3OD (a few drops), Me_4Si): 1.28 (4H, br s, $NCC(CH_2)_2$), 1.42 (4H, br s, $NC(CH_2)$ 2), 2.28 (2H, t, $FcCNCH_2$), 2.36 (2H, t, NCH_2), 3.56 (4H, s, NCH_2Ph), 3.58 (2H, s, $FcCH_2N$), 3.78 (2H, s, NCH_2Ph), 4.12 and 4.18 (5H, 4H, s each, Fc-H), 7.15–7.38, 7.86 (11H, 2H, m each, Ar-H). δ_C (75 MHz, $CDCl_3$ + CD_3OD (a few drops), Me_4Si): 22.7, 24.7, 25.6, 27.0, 31.6, 51.2, 52.0, 57.1, 60.4, 61.3, 68.4, 68.5, 68.6, 68.9, 70.6, 127.2, 127.3, 127.5, 128.2, 128.5, 129.6, 129.7, 129.9, 130.6, 130.9, 136.2, 136.5, 141.7, 141.8.

§ The observed DPV for **3 + 4** and **5** are available as ESI.† A decrease in current intensity with increasing D-glucose concentration was observed. Since reference compound **5** can not bind with D-glucose, the observed decrease in current intensity is due to a change in viscosity of the solution with added D-glucose. With compound **5** for concentrations of D-glucose greater than 0.1 mol dm^{-3} the current dramatically decreases due to high solution viscosity. Therefore, the ferrocene boronic acid sensors were titrated until a concentration of 0.1 mol dm^{-3} saccharide.

1 A. P. Davis and R. S. Wareham, *Angew. Chem., Int. Ed.*, 1999, **38**, 2979.
2 J. H. Hartley, T. D. James and C. J. Ward, *J. Chem. Soc., Perkin Trans. 1*, 2000, 3155.
3 T. D. James and S. Shinkai, *Top. Curr. Chem.*, 2002, **218**, 159.
4 S. Arimori, M. L. Bell, C. S. Oh, K. A. Frimat and T. D. James, *Chem. Commun.*, 2001, 1836.
5 S. Arimori, M. L. Bell, C. S. Oh, K. A. Frimat and T. D. James, *J. Chem. Soc., Perkin Trans. 1*, 2002, 802.
6 P. D. Beer, P. A. Gale and G. Z. Chen, *J. Chem. Soc., Dalton Trans.*, 1999, 1897.
7 W. Schuhmann and H.-L. Schmidt, *Adv. Biosensors*, 1992, **2**, 79.
8 A. Ori and S. Shinkai, *J. Chem. Soc., Chem. Commun.*, 1995, 1771.
9 A. N. J. Moore and D. D. M. Wayner, *Can. J. Chem.-Rev. Can. Chim.*, 1999, **77**, 681.
10 J. P. Lorand and J. D. Edward, *J. Org. Chem.*, 1959, **24**, 769.
11 The reference compound **3** has recently been prepared and proposed as a potentially active electrochemical unit by J. C. Norrild and I. Sotofte, *J. Chem. Soc., Perkin Trans 2*, 2002, 303.
12 A. Bard and L. Faulkner, *Electrochemical Methods*, 2nd edition, Wiley, New York, 2001.
13 The K values were analysed in KaleidaGraph using nonlinear (Levenberg–Marquardt algorithm) curve fitting. The errors reported are the standard errors obtained from the best fit.
14 C. R. Cooper and T. D. James, *J. Chem. Soc., Perkin Trans. 1*, 2000, 963.