Synthesis and characterisation of *N*-(3-dihydroxyborylphenyl)-5-mercaptopentanamide: a novel self-assembling vicinal diol receptor

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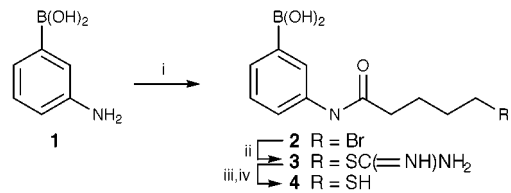
A novel self-assembling vicinal diol receptor, *N*-(3-dihydroxyborylphenyl)-5-mercaptopentanamide, has been synthesised *via* a three step procedure, characterised by NMR, mass spectoscopy and FTIR spectroscopy and shown to be functionally active by contact angle analysis, surface plasmon resonance and QCM.

The recent explosion in biosensor technology has led to the need for a greater variety of surface bound receptor systems. Thiol monolayer self-assembly is a technique of growing importance in many fields, particularly for the protection[1] or modification[2] of surfaces and the production of functionalised layers in sensor systems.[3–5]. The chemistry is versatile and robust, with a variety of headgroups attached to long-chain, thiol-terminated backbones. It has been suggested that the development of this technology may be hampered by a lack of new receptor systems.[6] Introduction of novel headgroup chemistry would continue to expand the uses of this technology. Here we report the synthesis of a new self-assembling vicinal diol receptor, based upon a thiol-modified boronic acid.

The interactions of sugars has been of interest to many with an increase in the use of boronic acids as receptors, however much of the work has taken place in free solution[7] or at air–water interfaces.[8] Boronic acids have long been known to interact with heavily hydroxylated species such as glycerol.[9] The semi-specific attachment point for vicinal diol groups, based upon the orientation of two adjacent hydroxy groups within a molecule, is covered in an excellent review by James *et al.*[10] Many saccharides contain hydroxy groups in the correct orientation and form stable cyclic esters when brought into contact with boronic acids.[11] Phenylboronic acids are known to form five-membered rings with *cis*-1,2-diol groups and less stable six-membered rings with the *trans*-isomer.[7] The synthesis of a self-assembling molecular layer capable of binding a wide variety of diol-containing moieties may open a route to producing a range of more specific multi-layer sensors.

Reaction of 3-aminophenylboronic acid **1** with 6-bromopentanoyl chloride in ethanolic solution (Scheme 1) yielded 3-(5-bromopentanoylamino)phenylboronic acid **2**. Further reaction with thiourea in methanolic solution over four days gave thioamidine **3**, which when treated with base prior to acidification was reduced to *N*-(3-dihydroxyborylphenyl)-5-mercaptopentanamide **4**.



Scheme 1 *Reagents and conditions*: i, 6-bromopentanoyl chloride, $NaHCO_3$, H_2O–EtOH, 25 °C, 2 h, stirred; ii, thiourea, MeOH, 25 °C, 4 days, stirred under N_2; iii, NaOH, MeOH, 25 °C, 4 days, stirred under N_2; iv, HCl.

Analysis of **4** by ^{1}H NMR† spectroscopy showed that > 85% of the sample was the desired thiol boronic acid. A small amount of the 3-aminophenylboronic acid starting material was also present. Further characterisation by LC-MS‡ and FTIR§ analysis gave molecular weight and structural information supporting the NMR data (Table 1).

Table 1 FTIR data for **4**

Position/cm^{-1}	Transmittance (%)	Assignment
3322	4.20	B–OH
2934	41.7	CH_2
2361	79.0	CH_3 (Possible S–H)
1663	3.20	*meta* substituted aromatic
1538	5.70	CH_2–NH
1344	5.80	C–N

The self-assembly characteristics of **4** were investigated by several techniques. Time resolved, sessile drop contact angle measurements of 2 μl water droplets were recorded on 38 nm gold films evaporated onto polished microscope slides. Initial contact angles for water droplets of 64 ± 3° (N = 6) were noted. Upon exposure to a 1 mM ethanolic solution of **4** in a clean environment, the contact angle was observed to decrease with time to 37 ± 2° (N = 6) after 2.5 h (Fig. 1). This is indicative of the rapid self-assembly mechanisms observed in simple long chain thiols on gold surfaces[12] and strongly suggested that surface modification had taken place *via* the widely accepted thiol–gold self-adsorption route,[13] the boronic acid headgroups rendering the surface more hydrophilic.

Characterisation of the self-assembly in real time using a Kretschmann configuration surface plasmon resonance (SPR) instrument, also employing 38 nm gold films as the SPR substrate, gave a 0.31º dual rate increase in SPR angle upon a 2.5 h exposure, at a concentration of the compound in ethanolic solution equal to that used for the contact angle measurements.

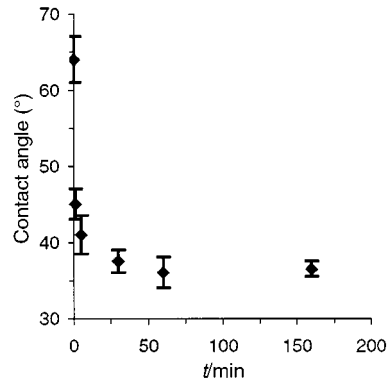


Fig. 1 Plot of sessile drop, water contact angle *vs.* time of immersion for a 38 nm gold coated microscope slide treated with a 1 mM ethanolic solution of **4** (droplet size 2 μl, T = 25 °C, N = 6 ± standard deviation).

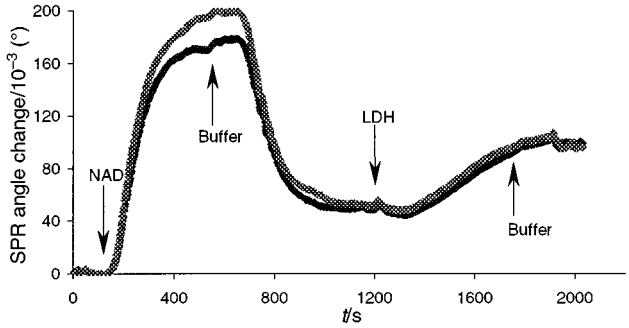


Fig. 2 SPR angle change for the binding of NAD (1 mg m^{-1}) to a gold substrate previously treated with **4** followed by lactate dehydrogenase (1×10^{-6} M) (flow rate = 4 μl min^{-1}, T = 30 °C, pH 7.2 Sorensons PBS).

Increases in SPR angle equate to material attachment to the substrate surface. Non-linear regression using a four parameter, double exponential rise relationship to the SPR data gave R = 0.99 and rate constants, $K1 = 1.1 \times 10^{-3}$ s^{-1} and $K2 = 6.91 \times 10^{-3}$ s^{-1}.The value of the measured $K1$ is of the order found by Debono *et al.*,[14] (4.3×10^{-3} s^{-1}) with SPR for the adsorption of dodecanethiol from ethanolic solution at an equal concentration. These results follow the distinct two step adsorption process found by DeBono *et al.*[14] and Bain *et al.*[12] The results show a very fast initial step lasting a few minutes giving rise to an 80–90% monolayer coverage, followed by a slower step lasting between minutes and hours depending upon the structure of the thiol, in which time the monolayer reorganises and completes its formation.

A similar two stage adsorption profile was observed upon the exposure of a 10 MHz lapped quartz crystal, thickness shear mode sensor with gold electrodes to a 1 mM ethanolic solution of the compound. A total frequency decrease of 126 Hz was recorded over an 8 h period for a single electrode in contact with the solution. The increased time required for the adsorption process to reach equilibrium is thought to be due to surface roughness; 2–3 μm troughs are found in the lapped crystal surface compared to the sub-micron polished surface used for SPR analysis.

Surface functionality of the adsorbed boronic acid SAM was further confirmed by contact angle and SPR experiments. Gold coated slides, previously exposed to the monolayer forming solution, with contact angles measuring 37 ± 2° (N = 6), were placed in a 1 mg ml^{-1} solution of nicotinamide adenine dinucleotide (NAD) in Sorenson's buffer, pH 7.2. Following a 15 min incubation at 30 °C the slides were removed, washed with further buffer, dried and the water contact angle measured as described previously. A further decrease in angle was recorded [27 ± 1.0° (N = 6)], suggesting that binding of the NAD to the surface may have occurred.

Real time analysis of NAD binding (Fig. 2) was carried out using SPR. NAD (1 mg ml^{-1}) in a buffer stream was passed over a boronic acid treated gold slide (4 μl s^{-1}). A residual SPR angle increase of 40 ± 2 millidegrees, (N = 3) verified that NAD had bound to the boronic acid layer. A similar profile was observed for lactose and maltose with SPR angle increases of 32 ± 3 (N = 3) and 24 ± 2° (N = 3) respectively. No residual change in SPR angle was observed when untreated gold surfaces were similarly challenged.

After treatment of the SPR slides with NAD, injections of lactate dehydrogenase (1×10^{-6}M, LDH), followed by a buffer wash were made. Fig. 2 shows strong binding to the NAD treated slides, with a residual SPR angle increase of 47 ± 3° (N = 3) and very little evidence of desorption. No overall change in SPR angle was observed for injection of LDH if the NAD was not present. This would support the binding of NAD *via* its diol groups to the surface, leaving the binding regions, at the adenine and nicotinamide units, available for LDH and a range of other dehydrogenases.[15]

In summary, this report describes a novel self-assembling boronic acid derivative with the potential to specifically interact with vicinal diol groups found within a range of nucleotides, saccharides, antibiotics and other systems. It has been shown that with a clean environment, formation of a correctly orientated layer on the surface is achieved and that vicinal diol containing species attach strongly only to the modified surface. Further, enzyme–cofactor binding has been demonstrated as part of a multilayer system, potentially opening new avenues for enzyme studies, *via* a relatively simple attachment route, applicable to a range of novel analysis tools such as SPR and QCM.

The authors wish to dedicate this paper to the memory of Dr Jim Baker who passed away prior to submission. We thank the EPSRC and SmithKline Beecham for financial support under the CASE award scheme, Award #G77.

Notes and references

† *Selected data* for **4**: δ_H[250 MHz, $CDCl_3$–DMSO-d_6 (1:1)]1.3 (CH_3CO_2H), 2.1, 2.3, 2.5 (CH_2), 3.0 (H_2O), 2.6, 3.4 (CH_2S), 7.1, 7.4, 7.6 (dd, C*H* aromatic), 9.0 (N*H*), 9.1 (CH_3CO_2H).

‡ LC/MS (Finnigan LCQ) yielded a single species of *m/z* (H^+) 254.300 at a retention time of 15.61 min comparable to the calculated mass of 254.101 ($C_{11}H_{16}BNO_3S$).

§ FTIR analysis on a Perkin-Elmer 1720 spectrophotometer, resolution 4 cm^{-1}, 20 scans.

1 L. Deng, M. Mrksich and G. Whitesides, *J. Am. Chem. Soc.*, 1996, **118**, 5136.
2 A. Ulman, *Chem. Rev.*, 1996, **96**, 1533 and references cited therin.
3 N. Nakashima and T. Taguchi, *Colloids Surf.*, 1995, **103**, 159.
4 K. T. Kinnear and H. G. Monbouquette, in *Biosensor and chemical sensor technology*, ACS, Washington, 1995, p. 82.
5 S. Storri, T. Santoni, M. Minunni and M. Mascini, *Biosens. Bioelectron.*, 1998, **13**, 347.
6 J. A. Riggs, R. K. Litchfield and B. D. Smith, *J. Org. Chem.*, 1996, **61**, 1148.
7 T. James, K. R. A. Samankumara Sansanayake and S. Shinkai, *Nature*, 1995, **374**, 345.
8 S. Shinkai, K. Tsukagoshi, Y. Ishikawa and T. Kunitake, *J. Chem. Soc., Chem. Commun.*, 1991, **15**, 1039.
9 L. Vignon, *Compt. Rend.*, 1874, **78**, 148.
10 T. D. James, K. R. A. Samankumara Sansanayake and S. Shinkai, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 1910.
11 T. Kuivila, *J. Org. Chem.*, 1954, **19**, 780.
12 C. D. Bain, E. B. Troughton, T. Y. Tao, J. Evall, G. M. Whitesides and R. G. Nuzzo, *J. Am. Chem. Soc.*, 1989, **111**, 321.
13 A. L. Plant, M. Brigham-Burke, E. C. Petrella and D. J. O'Shannessy, *Anal. Biochem.*, 1995, **226**, 342.
14 R. F. Debono, G. D. Loucks, D. D. Manna and U. J. Krull, *Can. J. Chem.*, 1996, **74**, 677.
15 L. Stryer, *Biochemistry*, 4th edn., W. H. Freeman, New York, 1995, p. 498.

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