Synthesis and evaluation of D-glucosamine-selective fluorescent sensors

Christopher R. Cooper and Tony D. James*

The University of Birmingham, School of Chemistry, Edgbaston, Birmingham, UK B15 2TT

Received (in Cambridge, UK) 18th November 1999, Accepted 2nd February 2000

Fluorescent PET (photoinduced electron transfer) sensors **11a** and **11b** with monoaza-18-crown-6 or monoaza-15-crown-5 ether and boronic acid receptor units are synthesised.

Much recent attention has been paid to the development of synthetic molecular receptors with the ability to recognise neutral organic species, including saccharides. A large majority of these systems have utilised hydrogen-bonding interactions for the purposes of recognition and binding of guest species. However, there is still no designed, monomeric receptor which can compete effectively with bulk water for low concentrations of monosaccharide substrates.[1]

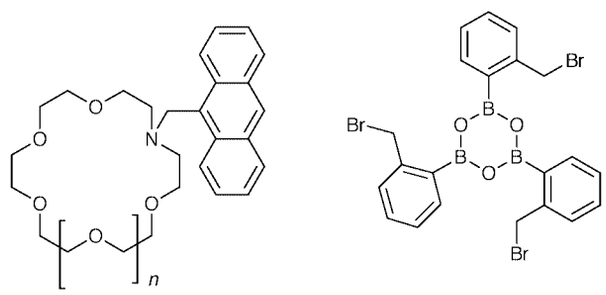
Boronic acids readily and reversibly form cyclic esters with diols in aqueous basic media. The most common interaction is with 1,2- and 1,3-diols of saccharides to form five- or six-membered rings respectively *via* two covalent bonds.

The complex stability increases from ethylene glycol to D-fructose, *i.e.* from the simple acyclic diols to the rigid, vicinal *cis*-diols of saccharides. This observed selectivity order is common to all monoboronic acids, not just to phenylboronic acid.[2]

In a series of recent papers the interaction of boronic acid and amine species[3–8] has been used to create photoinduced electron transfer (PET)[9–11] sensory systems for saccharides. The Lewis acidity of boronic acids is enhanced when they bind with a saccharide.[2] Therefore, the Lewis acid–base interaction between a boronic acid and a neighbouring tertiary amine is strengthened. The strength of this acid–base interaction modulates the PET from the amine (acting as a quencher) to anthracene (acting as a fluorophore). These compounds show increased fluorescence at neutral pH through suppression of the photoinduced electron transfer from nitrogen to anthracene on saccharide binding; a direct result of the stronger boron–nitrogen interaction[3–7]

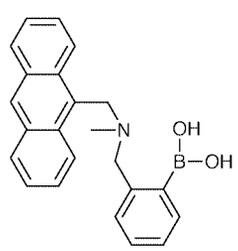
Previously it has been shown that two *identical* boronic acid binding units can be used in the selective recognition of glucose[4,8] and the chiral discrimination of monosaccharides.[5] Recent work by de Silva[12] and Shinkai[13,14] has shown how two *different* binding sites can be used to develop selective fluorescent sensors for γ-aminobutyric acid and D-glucuronic acid. The de Silva system consists of an azacrown ether (ammonium-binding site) and guanadinium (carboxylate-binding site). The Shinkai system consists of zinc(II) (carboxylate-binding site) and boronic acid (diol-binding site). These systems show how two different receptor sites can be used to obtain high selectivities for molecules containing multiple functional groups.

Over the last few years we have also been interested in developing systems containing two *different* binding sites. This article extends our preliminary work[15] with the synthesis of sensor **11a** and uses a more reliable method for the determination of the stability constants. We have also corrected an arithmetic error found in the earlier communication. Sensors **11a** and **11b** consist of monoaza-18-crown-6 ether or monoaza-15-crown-5 as a binding site for the ammonium terminal of D-glucosamine hydrochloride, while a boronic acid serves as a binding site for the diol (carbohydrate) part of D-glucosamine hydrochloride. The nitrogen of the azacrown ether unit can participate in PET with the anthracene fluorophore; ammonium ion binding can then cause fluorescence recovery. This recovery is due to hydrogen bonding from the ammonium ion to the nitrogen of the azacrown ether. The strength of this hydrogen-bonding interaction modulates the PET from the amine to anthracene. As explained above, the boronic acid unit can also participate in PET with the anthracene fluorophore, and diol binding can also cause fluorescence recovery. The anthracene unit serves as a rigid spacer between the two-receptor units, with the appropriate spacing for the glucose moiety. This system behaves like an **AND** logic gate,[16,17] in that fluorescence recovery is observed only when two chemical inputs are supplied; for this system the two chemical inputs are an ammonium cation and a diol group.



1 ($n = 0$)
2 ($n = 1$)

3



4

Results and discussion

Compounds **11a** and **11b** were synthesised as outlined in Scheme 1 (see below).

DOI: 10.1039/a909145i

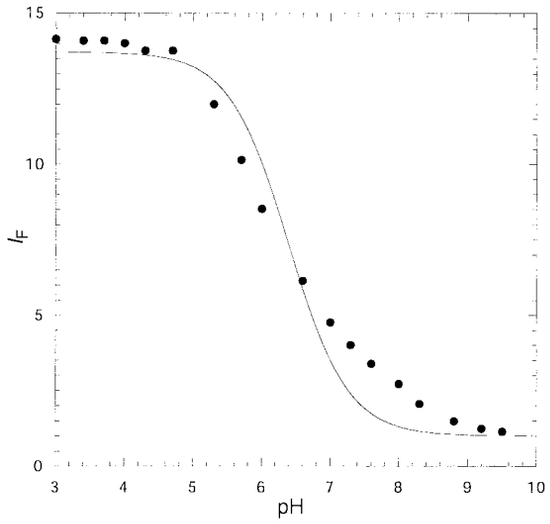


Fig. 1 Fluorescence intensity *versus* pH profile of **11a** at 25 °C; 3.10×10^{-6} mol dm^{-3} of **11a** in 0.05 mol dm^{-3} tetramethylammonium chloride in 33% methanol−67% water (w/w) solution.

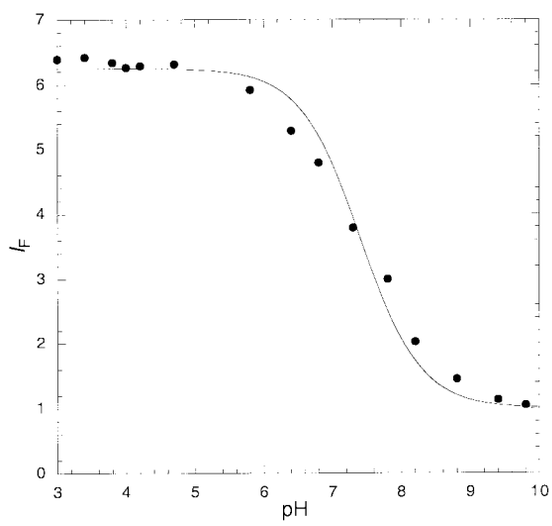


Fig. 2 Fluorescence intensity *versus* pH profile of **11b** at 25 °C; 3.33×10^{-6} mol dm^{-3} **11b** in 0.05 mol dm^{-3} tetramethylammonium chloride in 33% methanol–67% water (w/w) solution.

The fluorescence intensity *versus* pH of compounds **11a** and **11b** is shown in Fig. 1 and 2. The volume of 0.1 mol dm^{-3} hydrochloric acid *versus* pH of D-glucosamine hydrochloride is shown in Fig. 3. The curves in Fig. 1–3 were analysed[18] using equation (1) (see below). The pK_a of **11a** is 6.5 ± 0.1 and that of compound **11b** is 7.4 ± 0.1 [33% methanol–water (w/w), 0.05 mol dm^{-3} tetramethylammonium chloride]. The tetramethylammonium chloride present acts as an ionic buffer because small amounts of tetramethylammonium chloride are formed on adjustment of the pH with tetramethylammonium hydroxide and hydrochloric acid. Because the titrations are carried out in a methanol–water mixture rather than simply water, the concept of pH is not strictly applicable to this situation. However, for solutions in 50% methanol the pH is only changed by 0.1 of a pH unit compared with a 100% water solution.[19] The pK_a of D-glucosamine hydrochloride is 7.54 ± 0.01 (water, 0.05 mol dm^{-3} tetramethylammonium chloride). The pK_a-values of **1** and **2** have been previously determined, and are 8.1 and 8.5 respectively [25% methanol–water (v/v)].[20] The pK_a of **4** has also been previously determined as 2.9 (water, 0.05 mol dm^{-3} sodium chloride).[3,4] Interestingly the pK_a order of **11a** and **11b** mirrors that of **1** and **2**. The higher basicity of **2** as compared with **1** reflects the optimal complimentarity of the aza-18-crown-6 moiety with the hydronium ion, which has C_{3v} symmetry.

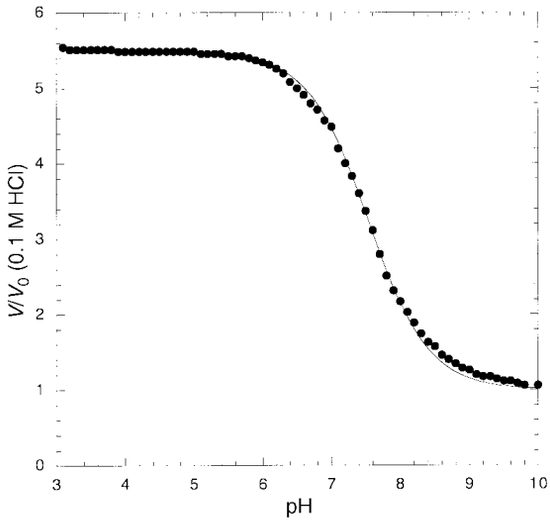


Fig. 3 Relative volume of HCl added *versus* pH profile of D-glucosamine hydrochloride at 25 °C; 5×10^{-2} mol dm^{-3} D-glucosamine hydrochloride in 0.05 mol dm^{-3} aq. tetramethylammonium chloride solution.

Table 1 Stability constant (K) for D-glucose and D-glucosamine hydrochloride with **1**, **2**, **4**, **11a** and **11b**

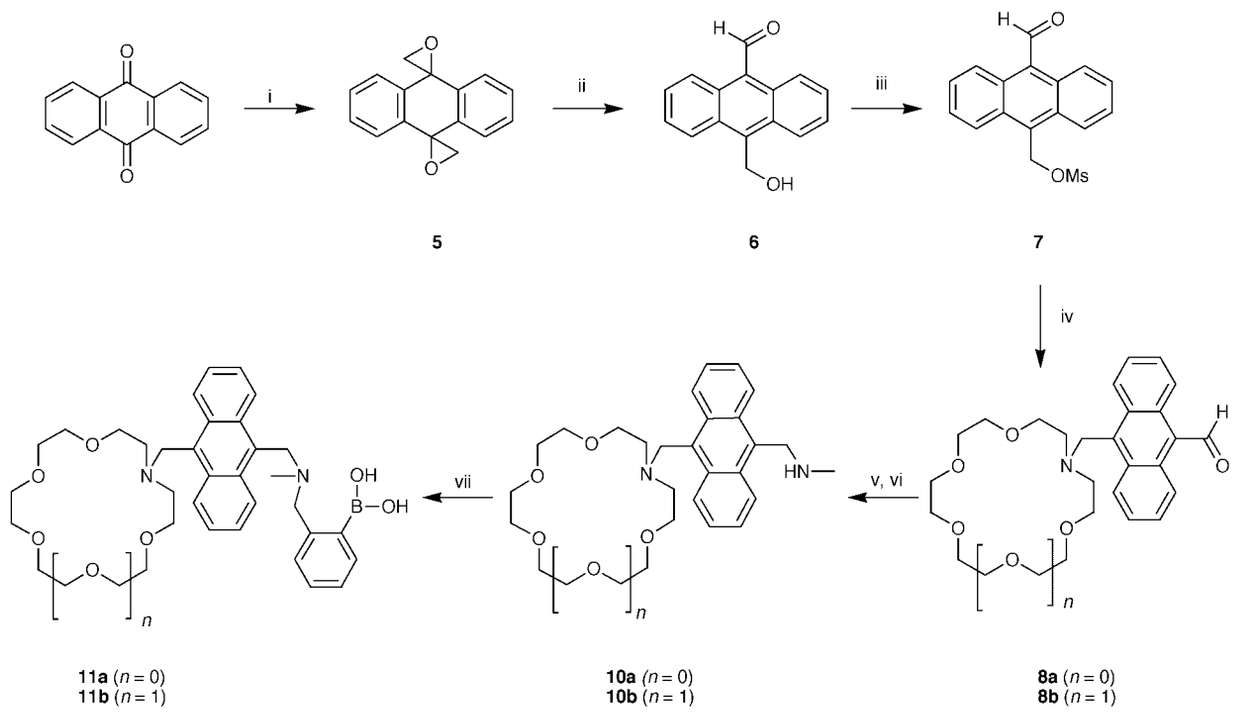
Sensor	K/mol dm^{-3}	
	D-Glucose	D-Glucosamine
1	[a]	[b]
2	[a]	[b]
4	67 ± 3	18 ± 1
11a	[b]	18 ± 2
11b	[b]	17 ± 2

[a] No fluorescence switching is possible. [b] No fluorescence change observed.

Binding studies were carried out in a 33.2% (w/w) ethanol–water buffer at pH 7.18.[21] As with methanol–water, the concept of pH is not strictly applicable to an ethanol–water mixture. However, for solutions in 50% ethanol the pH is only changed by 0.17 of a pH unit compared to a 100% water solution.[22] This buffer was chosen to balance the protonation of the azacrown ether and deprotonation of the D-glucosamine hydrochloride. At low pH the sensor is protonated and no signal will be observed; conversely at higher pH the D-glucosamine hydrochloride is present predominantly as free amine and will not bind strongly with the azacrown ether.

The stability constants for compounds **1**, **2**, **4**, **11a** and **11b** with D-glucose and D-glucosamine hydrochloride were calculated using equation (2) (see below) and are given in Table 1. The curves from which the constants were calculated are shown in Figs. 4–7.

Compounds **1** and **2** do not display any fluorescence enhancement with D-glucose because they have no saccharide-binding site. D-Glucosamine hydrochloride binding with compounds **1** and **2** is also not observed under the experimental conditions, since the buffer solution saturates with D-glucosamine hydrochloride before the binding event can be observed. As expected, compound **4** shows fluorescence enhancement with D-glucose ($K = 67 \pm 3$ mol dm^{-3}) and D-glucosamine hydrochloride ($K = 18 \pm 1$ mol dm^{-3}). With D-glucose the boronic acid has a choice of binding either the 1,2- or 4,6-diols, but with D-glucosamine hydrochloride, binding with just the 4,6-diol is possible. The stability constant of **4** with D-glucose is higher than that observed with D-glucosamine hydrochloride,



Scheme 1 Synthesis of boronic acid derivatives **11a** and **11b**. *Reagents and conditions (yields)*: i, NaH, $(CH_3)_3SI$, DMSO, rt (80%); ii, LiBr, CH_3CN, 60 °C (100%); iii, MsCl, Et_3N, DCM, rt (100%); iv, 1,4,7,10-tetraoxa-13-azacyclohexadecane or 1,4,7,10,13-pentaoxa-16-azacyclooctadecane, K_2CO_3, NaI, CH_3CN, reflux: **8a** (25%), **8b** (20%); v, MeOH, $MeNH_2$, rt: **9a** (94%), **9b** (100%); vi, $NaBH_4$, MeOH, rt: **10a** (100%), **10b** (100%); vii, **3**, K_2CO_3, CH_3CN, reflux: **11a** (62%), **11b** (79%).

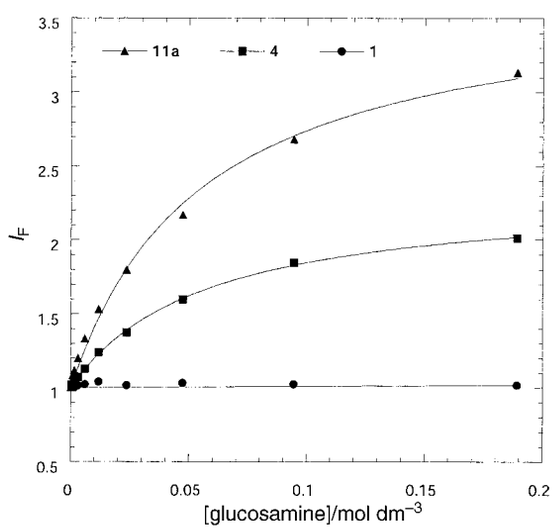


Fig. 4 Fluorescence intensity *versus* log [D-glucosamine hydrochloride] profile of (▲) **11a**, (■) **4** and (●) **1** at 25 °C; 3.75×10^{-6} mol dm^{-3} **11a**; 4.69×10^{-6} mol dm^{-3} **4**; 5.11×10^{-6} mol dm^{-3} **1** in 33.2% EtOH–66.8% H_2O, pH 7.18, λ_{ex} 372 nm, λ_{em} 425 nm.

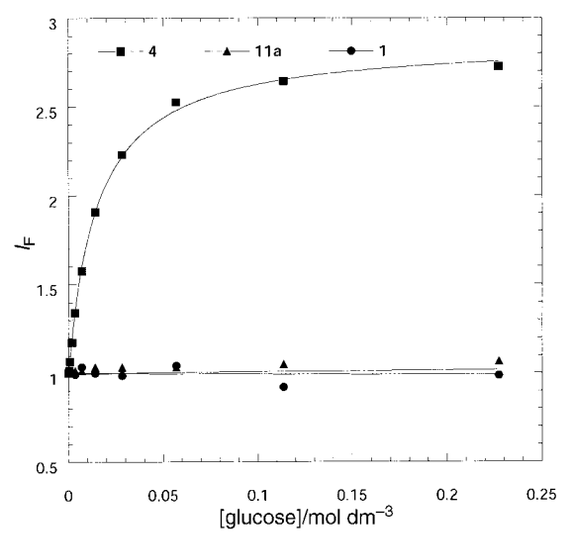


Fig. 5 Fluorescence intensity *versus* log [D-glucose] profile of (■) **4**, (▲) **11a** and (●) **1** at 25 °C; 4.69×10^{-6} mol dm^{-3} **4**; 3.75×10^{-6} mol dm^{-3} **11a**; 5.11×10^{-6} mol dm^{-3} **1** in 33.2% EtOH–66.8% H_2O, pH 7.18, λ_{ex} 372 nm, λ_{em} 425 nm.

reflecting the known selectivity of boronic acids for the 1,2-diol of D-glucose.[13] Compounds **11a** and **11b** show fluorescence increase with D-glucosamine hydrochloride ($K = 18 \pm 2$ mol dm^{-3} and 17 ± 2 mol dm^{-3} respectively), but no increase with D-glucose. This result clearly demonstrates that for a fluorescent output *both* a diol *and* ammonium group must be present in the guest. The stability of the D-glucosamine hydrochloride complex with compounds **11a**, **11b** and **4** is the same within experimental error ($K = 18 \pm 2$ mol dm^{-3}). These results show that the azacrown ether imparts no additional stability to the D-glucosamine complex. However, D-glucosamine must be involved in a hydrogen-bonding interaction with the secondary benzylic nitrogen of the azacrown ether in order to suppress PET. If such an interaction were not present then a fluorescence increase would not have been observed for compounds **11a** and **11b** with D-glucosamine hydrochloride. In our earlier communication of this work we reported a higher stability for **11b** ($K = 102$ mol dm^{-3})† than **4** with D-glucosamine ($K = 42$ mol dm^{-3}).† We now believe that the difference is a manifestation of the method of data analysis.

† Due to an arithmetic error the stability (K) values reported in ref. 15 are twenty times too high. Values reported previously: compound **4** with D-glucose log $K = 2.70$ ($K = 501$ mol dm^{-3}); compound **4** with D-glucosamine log $K = 2.92$ ($K = 832$ mol dm^{-3}); compound **11b** with D-glucosamine log $K = 3.31$ ($K = 2041$ mol dm^{-3}). Actual values: compound **4** with D-glucose log $K = 1.40$ ($K = 25$ mol dm^{-3}); compound **4** with D-glucosamine log $K = 1.62$ ($K = 42$ mol dm^{-3}); compound **11b** with D-glucosamine log $K = 2.01$ ($K = 102$ mol dm^{-3}).

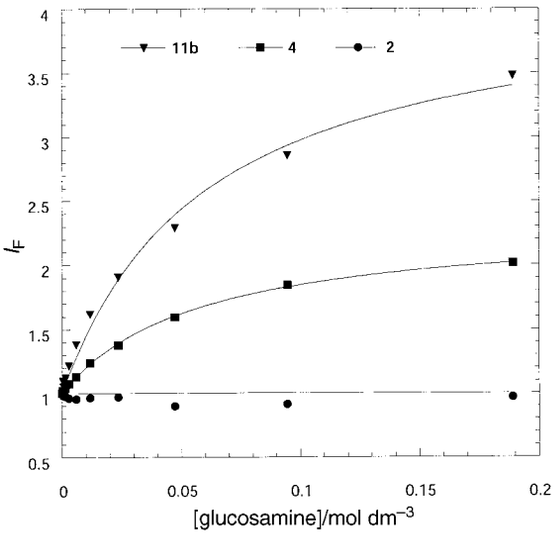


Fig. 6 Fluorescence intensity *versus* log [D-glucosamine hydrochloride] profile of (▼) **11b**, (■) **4** and (●) **2** at 25 °C; 3.33×10^{-6} mol dm^{-3} **11b**; 4.69×10^{-6} mol dm^{-3} **4**; 4.69×10^{-6} mol dm^{-3} **2** in 33.2% EtOH–66.8% H_2O, pH 7.18, λ_{ex} 372 nm, λ_{em} 425 nm.

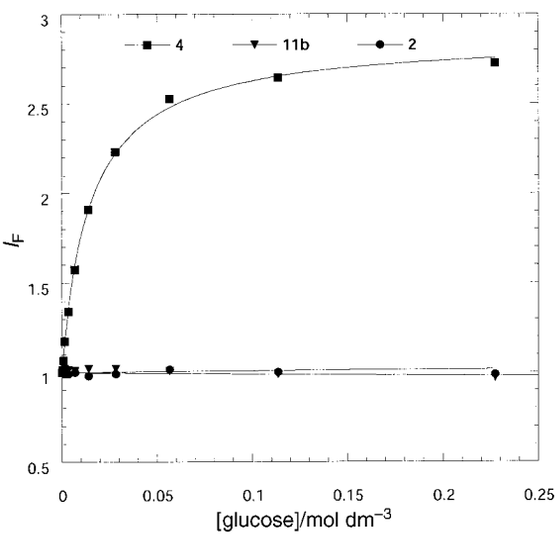


Fig. 7 Fluorescence intensity *versus* log [D-glucose] profile of (■) **4**, (▼) **11b** and (●) **2** at 25 °C; 4.69×10^{-6} mol dm^{-3} **4**; 3.33×10^{-6} mol dm^{-3} **11b**; 4.69×10^{-6} mol dm^{-3} **2** in 33.2% EtOH–66.8% H_2O, pH 7.18, λ_{ex} 372 nm, λ_{em} 425 nm.

The data were previously analysed using equation (3).[23] A plot of log [guest] *versus* log $[(I_F - I_{Fmin})/(I_{Fmax} - I_F)]$ gives an intercept of $-\log K$, with a slope of 1 (if the slope significantly deviates from 1 the relationship is invalid). Data analysis using equation (3) works well when the final fluorescence intensity (I_{Fmax}) is known. However, with the D-glucosamine titration a true final fluorescence intensity (I_{Fmax}) may have not been obtained. The reason for the system not obtaining maximum fluorescence intensity at high D-glucosamine concentrations may be due to two factors, (1) the ionic strength of the solution or (2) insolubility of the D-glucosamine.

We therefore decided to calculate the stability constant (K) using equation (2), which does not require the final fluorescence intensity (I_{Fmax}). Curve fitting the fluorescence intensity (I_f) *versus* the concentration of guest (both I_{Fmax} and K were varied) gives the calculated stability constant (K) and final fluorescence intensity (I_{Fmax}). In order to eliminate any high-concentration effects the maximum concentration of guest added was 0.25 mol dm^{-3}. We believe that the stability constants (K) obtained using equation (2) and shown in Table 1 are closer to the true values than those previously determined using equation (3).

Data analysis

$$I_F = (I_{Fmin} + I_{Fmax} \times K \times [H^+])/(1 + K \times [H^+]) \quad (1)$$

$$I_F = (I_{Fmin} + I_{Fmax} \times K \times [\text{guest}])/(1 + K \times [\text{guest}]) \quad (2)$$

$$\log [\text{guest}] = \log [(I_F - I_{Fmin})/(I_{Fmax} - I_F)] - \log K \quad (3)$$

I_{Fmin} is the initial (mimimum) fluorescence intensity; I_{Fmax} is the final (maximum) fluorescence intensity; I_F is the fluorescence intensity for a particular guest concentration; K is the stability constant of the receptor with the guest; $[H^+]$ is the concentration of protons; [guest] is the concentration of the guest.

Both pK_a and stability-constant (K) curves were analysed in KaleidaGraph‡ using nonlinear (Levenberg–Marquardt algorithm) curve fitting of equations (1) and (2). The errors reported are the standard errors ($\pm\sigma/\sqrt{N}$) obtained from the best fit.

Conclusions

The results with compounds **11a** and **11b** show how it is possible to develop simple **AND** logic gates. In these systems fluorescence recovery is observed only when two chemical inputs are supplied; the two chemical inputs are an ammonium cation and a diol group. Interestingly this work has shown that the second binding site, the azacrown ether, is required for the switching mechanism. However, the azacrown ether imparts no additional stability to the D-glucosamine–receptor complex since the stability of the D-glucosamine complex with **4**, **11a** and **11b** is the same. This result is not unexpected since it is well known that ammonium ions only weakly bind with crown ethers in aqueous solution.[24]

With this work we have demonstrated that the boronic acid PET system can be used in combination with other binding sites to create new and selective receptors for important biological molecules. We believe that this unit will be increasingly used as a general building block in molecular receptor design.

Experimental

General procedures

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-300 (300.13 and 75.47 MHz respectively) spectrometer. All spectra were recorded relative to tetramethylsilane as the internal standard. The multiplicities of the spectroscopic data are presented in the following manner; s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. J-Values are given in Hz.

Chemical ionisation (CI) and electron impact (EI) mass spectra were recorded on a VG ProSpec mass spectrometer. CI methods used ammonia as the reagent gas. Liquid secondary ion (LSI) mass spectra were recorded using a VG Zabspec instrument. A Micromass LCT mass spectrometer was used for both low-resolution electrospray time of flight (ES-TOF) mass spectra (using a methanol mobile phase) and high-resolution mass spectra (HRMS) measurements (using a lockmass incorporated into the mobile phase). HRMS measurements were also obtained from either the VG ProSpec or VG Zabspec spectrometers.

Elemental analyses were performed at the University of North London.

‡ KaleidaGraph Version 3.08d for the Macintosh, published by Synergy Software and developed by Abelbeck Software, 2457 Perikiomen Avenue, Reading, PA 19606. A user-defined curve fit $[1 + m2 \times m1 \times (M0)]/(1 + m1 \times (M0)]$ derived from equations (1) and (2) was used in all calculations. The initial value of $m1$ (K) was set to 1 and the initial value of $m2$ (I_{Fmax}) was set to 1.1. The variable ($M0$) was $[H^+]$ for equation (1) and [guest] for equation (2). The allowable error was set to 0.1%. For all curves the coefficient of determination (r^2) was ≥0.99.

Thin-layer chromatography (TLC) was performed on pre-coated aluminium-backed silica gel plates supplied by E. Merck, AG, Darmstadt, Germany (Silica gel 60 F254, thickness 0.2 mm, Art. 5554). Visualisation was achieved by UV light (254 nm). Gravity column chromatography was performed on silica gel (E. Merck A.G. Kieselgel 60, Art. 7734). Column fractions were collected, and monitored by TLC.

Dichloromethane and acetonitrile were both dried by refluxing with calcium hydride. They were subsequently distilled, and collected by dry syringe as required. Methanol was dried by refluxing with iodine and magnesium turnings for 3 h, and subsequently distilled onto 3 Å molecular sieves. Triethylamine was distilled from calcium hydride and stored under an atmosphere of nitrogen over calcium hydride. All other reagents and solvents were used as supplied by the Aldrich Chemical Co. Ltd., Lancaster Synthesis Ltd. and Fisher Scientific Ltd.

13-(9-Anthrylmethyl)-13-aza-1,4,7,10-tetraoxacyclopentadecane 1 and 16-(9-anthrylmethyl)-16-aza-1,4,7,10,13-pentaoxaxcyclooctadecane 2

Prepared according to literature procedure.[20]

2,4,6-Tris[*o*-(bromomethyl)phenyl]boroxine 3

Prepared according to literature procedure.[25]

N-(9-Anthrylmethyl)-*N*-[*o*-(dihydroxyboryl)benzyl]methylamine 4

Prepared according to literature procedure.[4]

9′,10′-Dihydrodispiro[oxirane-2,9′-anthracene-10′,2″-oxirane] 5 and 10-(hydroxymethyl)anthracene-9-carbaldehyde 6

Prepared according to literature procedure.[26]

(10-Formyl-9-anthryl)methyl methanesulfonate 7

Methanesulfonyl chloride (0.41 cm^3, 7.62 mmol) as a solution in dry dichloromethane (20 cm^3) was added over a period of 30 min to a stirred solution of 10-(hydroxymethyl)anthracene-9-carbaldehyde **6** (900 mg, 3.81 mmol) and triethylamine (2.50 cm^3, 17.9 mmol) in 50 cm^3 of dry dichloromethane, under an argon atmosphere. The reaction mixture was then stirred for a further 50 h at room temperature in the dark. After this time, water (150 cm^3) was added to the reaction mixture, the organic layer was removed, and the aqueous layer extracted with dichloromethane (5 × 50 cm^3). The organic extracts were combined, dried ($MgSO_4$), and concentrated under reduced pressure to afford the *mesyl ester* **7** as a yellow solid (1.29 g, 100%), mp 162–164 °C (decomp.); ν_{max}(Nujol)/cm^{-1} 1734 (s, C=O), 1376 (s, S=O); δ_H (300 MHz; C^2HCl_3) 1.55 (3H, s, CH_3), 5.60 (2H, s, CH_2), 7.70 (4H, m, 2-, 3-, 6-, 7-ArH), 8.35 (2H, m, 1-, 8-ArH), 8.85 (2H, m, 4-, 5-ArH), 11.51 (1H, s, CHO); δ_C (75 MHz; C^2HCl_3), 56.0 (CH_3), 66.8 (CH_2), 124.0 (9-, 10-ArCH), 124.2 (2-, 7-ArCH), 125.2 (3-, 6-ArCH), 126.5 (1-, 8-ArCH), 128.4 (4-, 5-ArCH), 130.6 (8a-, 9a-ArC), 131.4 (4a-, 10a-ArC), 193.9 (CHO); *m*/*z* (EI) 299 (40%, $[M - CH_3]^+$), 219 (25, $[M - OSO_2CH_3]^+$), 191 (100, $[M - OSO_2CH_3 - CO]^+$); R_f 0.60 (hexane–ethyl acetate, 2:1).

10-[(1-Aza-4,7,10,13-tetraoxacyclopentadecyl)methyl]-anthracene-9-carbaldehyde 8a

Potassium carbonate (460 mg, 3.33 mmol) and sodium iodide (67 mg, 40 mol%, catalytic) were added to a stirred solution of mesyl ester **7** (350 mg, 1.11 mmol) in dry acetonitrile (25 cm^3). This was followed by the addition of 1,4,7,10-tetraoxa-13-azacyclopentadecane (60 mg, 0.36 mmol). The reaction mixture was then stirred under reflux, in an atmosphere of argon, for 16 h. After this time, TLC indicated that consumption of the starting material had occurred and the solvent was removed under reduced pressure. Water was added (50 cm^3) and the aqueous layer extracted with chloroform (3 × 50 cm^3). The organic extracts were combined, dried ($MgSO_4$), and concentrated under reduced pressure. The residual yellow gum was purified by silica chromatography (chloroform–methanol, 15:1) to give *aldehyde* **8a** as a yellow solid (122 mg, 25%), mp 163–165 °C (decomp.); ν_{max}(Nujol)/cm^{-1} 1730 (s, C=O) (Found: C, 71.51; H, 6.97; N, 3.21. $C_{26}H_{31}NO_5$ requires C, 71.40; H, 7.10; N, 3.20%); δ_H (300 MHz; C^2HCl_3) 2.90 (4H, t, $^3J_{H-H}$ 5.0, 2 × NCH_2CH_2), 3.55–3.70 (16H, m, 8 × OCH_2), 4.59 (2H, s, NCH_2), 7.51–7.65 (4H, m, 2-, 3-, 6-, 7-ArH), 8.65 (2H, d, $^3J_{H-H}$ 8.9, 1-, 8-ArH), 8.86 (2H, d, $^3J_{H-H}$ 8.9, 4-, 6-ArH), 11.45 (1H, s, CHO); δ_C (75 MHz; C^2HCl_3), 54.2 (CH_2), 69.8–71.5 (2 × NCH_2CH_2 and 8 × OCH_2), 123.8 (2-, 7-ArCH), 125.7 (3-, 6-ArCH), 126.0 (1-, 8-ArCH), 128.2 (4-, 5-ArCH), 130.8 (8a-, 9-, 9a-ArC), 131.1 (4-, 4a-, 10a-ArC), 193.9 (CHO); *m*/*z* (LSI-MS) 438 (70% $[M + H]^+$), 219 (100, $[M - \text{azacrown}]^+$); R_f 0.43 (chloroform–methanol, 15:1).

13-{10-[(1*E*)-2-Azaprop-1-enyl]-9-anthrylmethyl}-13-aza-1,4,7,10-tetraoxacyclopentadecane 9a

Methylamine (1 cm^3; 2.0 mol dm^3 in methanol) was added to a solution of aldehyde **8a** (68 mg, 0.16 mmol) in dry methanol (2 cm^3). The reaction mixture was then stirred at room temperature for 16 h in an atmosphere of argon. After this time the reaction mixture was concentrated under reduced pressure to afford imine **9a** as a stiff yellow gum which was used in the next step without further purification (68 mg, 94%); R_f 0.28 (chloroform–methanol, 15:1).

Preparation of 9-methylaminomethyl-10-[(1,4,7,10-tetraoxa-13-azacyclopentadecyl)methyl]anthracene 10a

Sodium borohydride (17 mg, 0.45 mmol) was added in one batch to a solution of imine **9a** (68 mg, 0.15 mmol) in dry methanol (5 cm^3). The reaction mixture was then stirred at room temperature for 5 h. After this time, TLC indicated that consumption of the starting material had occurred and the solvent was removed under reduced pressure. Water (25 cm^3) was added and the aqueous layer extracted with chloroform (3 × 25 cm^3). The organic extracts were combined, dried ($MgSO_4$), and concentrated under reduced pressure. The resultant yellow gum was purified by precipitation from chloroform using hexane to give *amine* **10a** as a yellow solid (69 mg, 100%), mp 140–143 °C (decomp.) (HRMS Found: $[M + Na]^+$, 475.2570. $C_{27}H_{36}N_2NaO_4$ requires *m*/*z*, 475.2573) (Found: C, 71.67; H, 8.11; N, 6.23. $C_{27}H_{36}N_2O_4$ requires C, 71.65; H, 8.02; N, 6.19%); δ_H (300 MHz; C^2HCl_3) 2.66 (3H, s, CH_3), 2.91 (4H, t, $^3J_{H-H}$ 5.0, 2 × NCH_2CH_2), 3.55–3.68 (16H, m, 8 × OCH_2), 4.60 (2H, s, $NHCH_2Ar$), 4.65 (2H, s, NCH_2Ar), 7.47–7.55 (4H, m, 2-, 3-, 6-, 7-ArH), 8.34 (2H, m, 4-, 5-ArH), 8.57 (2H, m, 1-, 8-ArH); δ_C (75 MHz; C^2HCl_3), 37.0 (CH_3), 47.8 ($NHCH_2Ar$), 52.6 (NCH_2Ar), 70.0–70.9 (2 × NCH_2CH_2 and 8 × OCH_2), 124.5 (2-, 7-ArCH), 125.1 (3-, 6-ArCH), 125.6 (4-, 5-ArCH), 126.0 (1-, 8-ArCH), 130.0 (8a-, 9a-ArC), 130.9 (9-ArC), 131.2 (4a-, 10a-ArCH), 131.8 (10-ArC); *m*/*z* (LSI-MS) 453 (100%, $[M + H]^+$); R_f 0.15 (chloroform–methanol, 15:1).

N-({10-[(1-Aza-4,7,10,13-tetraoxacyclopentadecyl)methyl]-9-anthryl}methyl)-*N*-[*o*-(dihydroxyboryl)benzyl])methylamine 11a

Compound **3** (52 mg, 81 μmol) and potassium carbonate (152 mg, 1.11 mmol) were added to a stirred solution of amine **10a** (100 mg, 0.22 mmol) in dry acetonitrile (15 cm^3). The reaction mixture was then stirred under reflux, in an argon atmosphere, for 5 h. After this time, TLC indicated that consumption of the starting material had occurred and the solvent was removed under reduced pressure. Water was added (25 cm^3) and the aqueous layer extracted with chloroform (3 × 25 cm^3). The organic extracts were combined, dried ($MgSO_4$), and concen-

trated under reduced pressure. The resultant yellow gum was then precipitated from chloroform using hexane to give *boronic acid* **11a** as a yellow solid (80 mg, 62%), mp 109–111 °C (HRMS Found: $[M + Na]^+$, 609.3118. $C_{34}H_{43}BN_2NaO_6$ requires *m*/*z*, 609.3112) (Found: C, 69.21; H, 7.23; N, 4.59. $C_{34}H_{43}BN_2O_6$ requires C, 69.61; H, 7.39; N, 4.78%); δ_H (300 MHz; $C^2H_3O^2H$) 2.26 (3H, s, CH_3), 2.83 (4H, t, $^3J_{H–H}$ 5.5, 2 × NCH_2CH_2), 3.40–3.68 (16H, m, 8 × OCH_2), 4.10 (2H, s, $ArCH_2NCH_2Ar'CH_2N$), 4.52 (2H, s, $ArCH_2NCH_2Ar'CH_2N$), 4.68 (2H, s, $ArCH_2NCH_2Ar'CH_2N$), 7.29–7.36 (3H, m, 4-, 5-, 6-ArH), 7.38–7.50 (4H, m, 2-, 3-, 6-, 7-Ar′H), 7.80 (1H, d, $^3J_{H–H}$ 6.6, 3-ArH), 8.06 (2H, d, $^3J_{H–H}$ 8.5, 4-, 5-Ar′H), 8.53 (2H, d, $^3J_{H–H}$ 8.50, 1-, 8-Ar′H); δ_C (75 MHz; $C^2H_3O^2H$) 41.9 (CH_3), 53.2 ($ArCH_2NCH_2Ar'CH_2N$), 54.1 ($ArCH_2NCH_2Ar'CH_2N$), 55.9 ($ArCH_2NCH_2Ar'CH_2N$), 70.1–71.0 (2 × NCH_2CH_2 and 8 × OCH_2), 125.1 (6-ArCH), 125.4 (5-ArCH), 125.9 (4-ArCH), 127.2 (3-, 6-Ar′CH), 127.4 (2-, 7-Ar′CH), 127.7 (4-, 5-Ar′CH), 130.1 (1-, 8-Ar′CH), 130.9 (10-Ar′C), 131.0 (9-Ar′C), 134.1 (3-ArCH); *m*/*z* (ES^+) 609 (85%, $[M + Na]^+$), 587 (65, $[M + H]^+$), 368 (100, $[M - azacrown]^+$); R_f 0.22 (chloroform–methanol, 15 : 1).

10-[(1-Aza-4,7,10,13,16-pentaoxocyclooctadecyl)methyl]anthracene-9-carbaldehyde 8b

Potassium carbonate (50 mg, 0.36 mmol) and sodium iodide (10 mg, 40 mol%, catalytic) were added to a stirred solution of mesyl ester **7** (50 mg, 0.16 mmol) in dry acetonitrile (10 cm^3). This was followed by addition of 1,4,7,10,13-pentaoxa-16-azacyclooctadecane (60 mg, 0.36 mmol). The reaction mixture was then stirred under reflux, in an atmosphere of argon, for 16 h. After this time, TLC indicated that consumption of the starting material had occurred and the solvent was removed. Water (25 cm^3) was added and the aqueous layer extracted with chloroform (3 × 25 cm^3). The organic extracts were combined, dried ($MgSO_4$), and concentrated under reduced pressure. The residual yellow gum was purified by silica chromatography using chloroform–methanol (15 : 1) to give *aldehyde* **8b** as a yellow solid (20 mg, 20%), mp 143–145 °C (decomp.) (HRMS Found: $[M + Na]^+$, 504.2344. $C_{28}H_{35}NNaO_6$ requires *m*/*z*, 504.2362); ν_{max}(Nujol)/cm^{-1} 1734 (s, C=O) (Found: C, 70.02; H, 7.35; N, 2.72. $C_{28}H_{35}NO_6$ requires C, 69.85; H, 7.28; N, 2.91%); δ_H (300 MHz; C^2HCl_3) 2.85 (4H, t, $^3J_{H–H}$ 5.0, 2 × NCH_2CH_2), 3.55–3.70 (20H, m, 10 × OCH_2), 4.65 (2H, s, NCH_2Ar), 7.50–7.70 (4H, m, 2-, 3-, 6-, 7-ArH), 8.70 (2H, d, $^3J_{H–H}$ 9.0, 1-, 8-ArH), 8.90 (2H, d, $^3J_{H–H}$ 9.0, 4-, 5-ArH), 11.50 (1H, s, CHO); δ_C (75 MHz; C^2HCl_3), 53.8 (NCH_2Ar), 70.2–70.8 (2 × NCH_2CH_2 and 10 × OCH_2), 125.0 (2-, 7-ArCH), 125.2 (3-, 6-ArCH), 125.3 (1-, 8-ArCH), 125.8 (4-, 5-ArCH), 129.6 (8a-, 9-, 9a-ArC), 131.2 (4-, 4a-, 10a-ArC), 212.9 (CHO); *m*/*z* (LSI-MS) 482 (100%, $[M + H]^+$); R_f 0.46 (chloroform–methanol, 15 : 1).

16-{10-[(1*E*)-2-Azaprop-1-enyl]-9-anthrylmethyl}-16-aza-1,4,7,10,13-pentaoxacyclooctadecane 9b

Methylamine (5 cm^3; 2.0 mol dm^{-3} in methanol) was added to a solution of aldehyde **8b** (43 mg, 90 μmol) in dry methanol (3 cm^3). The reaction mixture was then stirred at room temperature for 16 h in an atmosphere of argon. After this time the reaction mixture was concentrated under reduced pressure to afford imine **9b** as a stiff yellow gum which was used for the next step without further purification (44 mg, 100%); R_f 0.29 (chloroform–methanol, 15 : 1).

N-({10-[(1-Aza-4,7,10,13,16-pentaoxacyclooctadecyl)methyl]-9-anthryl}methyl)methylamine 10b

Sodium borohydride (10 mg, 0.24 mmol) was added in batches to a solution of imine **9b** (44 mg, 80 μmol) in dry methanol (5 cm^3). The reaction mixture was then stirred at room temperature for a further 4 h. After this time, TLC indicated that consumption of the starting material had occurred and the solvent was removed under reduced pressure. Water (25 cm^3) was added and the aqueous layer extracted with chloroform (3 × 25 cm^3). The organic extracts were combined, dried ($MgSO_4$), and concentrated under reduced pressure to afford amine **10b** as a yellow solid (45 mg, 100%), mp 145–147 °C (decomp.) (HRMS Found: $[M + Na]^+$, 519.2833. $C_{29}H_{40}N_2NaO_5$ requires *m*/*z*, 519.2835); δ_H (300 MHz; C^2HCl_3) 2.65 (3H, s, CH_3), 2.91 (4H, t, $^3J_{H–H}$ 5.0, 2 × NCH_2CH_2), 3.55–3.70 (20H, m, 10 × OCH_2), 4.60 (2H, s, $NHCH_2Ar$), 4.65 (2H, s, NCH_2Ar), 7.45–7.55 (4H, m, 2-, 3-, 6-, 7-ArH), 8.35 (2H, dd, $^3J_{H–H}$ 9.0, $^4J_{H–H}$ 2, 4-, 5-ArH), 8.60 (2H, dd, $^3J_{H–H}$ 9.0, $^4J_{H–H}$ 2, 1-, 8-ArH); δ_C (75 MHz; C^2HCl_3) 36.1 (CH_3), 51.8 ($NHCH_2Ar$), 53.8 (NCH_2Ar), 70.2–70.8 (2 × NCH_2CH_2 and 10 × OCH_2), 125.0 (3-, 6-ArCH), 125.2 (2-, 7-ArCH), 125.3 (4-, 5-ArCH), 125.8 (1-, 8-ArCH), 129.6 (4-, 4a-, 10a-ArC), 131.2 (8a-, 9-, 9a-ArC); *m*/*z* (LSI-MS) 482 (100%, $[M + H]^+$); R_f 0.22 (chloroform–methanol, 15 : 1).

N-({10-[(1-Aza-4,7,10,13,16-pentaoxacyclooctadecyl)methyl]-9-anthryl}methyl)-*N*-[*o*-(dihydroxyboryl)benzyl])methylamine 11b

Compound **3** (15 mg, 25.1 μmol) and potassium carbonate (30 mg, 0.21 mmol) were added to a stirred solution of amine **10b** (37 mg, 70 μmol) in dry acetonitrile (5 cm^3). The reaction mixture was then stirred under reflux in argon for 4 h. After this time, TLC indicated that consumption of the starting material had occurred and the solvent was removed under reduced pressure. Water (25 cm^3) was added and the aqueous layer extracted with chloroform (3 × 25 cm^3). The organic extracts were combined, dried ($MgSO_4$), and concentrated under reduced pressure. The residual yellow gum was then precipitated from chloroform using hexane to give boronic acid **11b** as a yellow powder (35 mg, 79%), mp 112–114 °C (HRMS Found: $[M + Na]^+$, 653.3367. $C_{36}H_{47}BN_2NaO_7$ requires *m*/*z*, 653.3374); δ_H (300 MHz; $C^2H_3O^2H$) 2.39 (3H, s, CH_3), 2.84 (4H, t, $^3J_{H–H}$ 5.1, 2 × NCH_2CH_2), 3.45–3.65 (20H, m, 10 × OCH_2), 4.33 (2H, s, $ArCH_2NCH_2Ar'CH_2N$), 4.64 (2H, s, $ArCH_2NCH_2Ar'CH_2N$), 5.00 (2H, s, $ArCH_2NCH_2Ar'CH_2N$), 7.30 (1H, d, $^3J_{H–H}$ 6.7, 6-ArH), 7.37 (2H, t, $^3J_{H–H}$ 6.7, 4-, 5-ArH), 7.50–7.60 (4H, m, 2-, 3-, 6-, 7-Ar′H), 7.74 (1H, d, $^3J_{H–H}$ 6.7, 3-ArH), 8.17 (2H, d, $^3J_{H–H}$ 8.5, 4-, 5-Ar′H), 8.65 (2H, d, $^3J_{H–H}$ 8.5, 1-, 8-Ar′H); δ_C (75 MHz; $C^2H_3O^2H$) 50.7 ($ArCH_2NCH_2Ar'CH_2N$), 51.0 ($ArCH_2NCH_2Ar'CH_2N$), 51.3 (CH_3), 53.1 ($ArCH_2NCH_2Ar'CH_2N$), 69.1–69.7 (2 × NCH_2CH_2 and 10 × OCH_2), 123.5 (6-ArCH), 124.1 (5-ArCH), 124.4 (4-ArCH), 125.2 (3-, 6-Ar′CH), 126.9 (2-, 7-Ar′CH), 129.5 (4-, 5-Ar′CH), 133.2 (1-, 8-Ar′CH), 133.6 (3-ArCH), 130.2 (4a-, 8a-, 9a-, 10a-Ar′C), 130.3 (9-, 10-Ar′C); *m*/*z* (ES^+) 669 (5%, $[M + K]^+$), 653 (20, $[M + Na]^+$), 631 (100, $[M + H]^+$); R_f 0.19 (chloroform–methanol, 15 : 1).

pH Titration of 11a and 11b

The fluorescence emission spectra of **11a** (3.10×10^{-6} mol dm^{-3}) and **11b** (3.33×10^{-6} mol dm^{-3}) in a 0.05 mol dm^{-3} tetramethylammonium chloride solution in 33% methanol–67% water (w/w) were recorded as the pH was changed from pH 3 to 10 in approximate intervals of 0.5 pH units. The pH was controlled using minimum volumes of tetramethylammonium hydroxide and hydrochloric acid solutions.

D-Glucose titration of 1, 2, 4, 11a and 11b at pH 7.18

The fluorescence spectra of **1** (5.11×10^{-6} mol dm^{-3}), **2** (4.69×10^{-6} mol dm^{-3}), **11a** (3.75×10^{-6} mol dm^{-3}), **11b** (3.33×10^{-6} mol dm^{-3}), **4** (4.69×10^{-6} mol dm^{-3}) in a pH 7.18 buffer [0.002 14 mol dm^{-3} triethanolamine, 0.004 23 mol dm^{-3} triethanolamine hydrochloride, in 33% ethanol–67% water

(w/w)] were recorded as increasing amounts of D-glucose were added to the solution. For all titrations the observed pH fluctuated by less than ±0.1 units from the buffered pH.

D-Glucosamine hydrochloride titration of 1, 2, 4, 11a and 11b at pH 7.18

The previous experiment was repeated but with increasing amounts of D-glucosamine hydrochloride added to the solution. For all titrations the observed pH fluctuated by less than ±0.1 units from the buffered pH.

Acknowledgements

T. D. J. wishes to acknowledge the Royal Society for support through the award of a University Fellowship. C. R. C. wishes to acknowledge the School of Chemistry (University of Birmingham) for support through the award of a School Studentship. T. D. J. and C. R. C. would also like to thank Mr Christopher J. Ward for preparing compound **3**.

References

1 C. J. Davis, P. T. Lewis, M. E. McCarroll, M. W. Read, R. Cueto and R. M. Strongin, *Org. Lett.*, 1999, **1**, 331.
2 J. P. Lorand and J. O. Edwards, *J. Org. Chem.*, 1959, **24**, 769.
3 T. D. James, K. R. A. S. Sandanayake and S. Shinkai, *J. Chem. Soc., Chem. Commun.*, 1994, 477.
4 T. D. James, K. R. A. S. Sandanayake, R. Iguchi and S. Shinkai, *J. Am. Chem. Soc.*, 1995, **117**, 8982.
5 T. D. James, K. R. A. S. Sandanayake and S. Shinkai, *Nature*, 1995, **374**, 345.
6 T. D. James, K. R. A. S. Sandanayake and S. Shinkai, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 1911.
7 T. D. James, P. Linnane and S. Shinkai, *Chem. Commun.*, 1996, 281.
8 T. D. James, K. R. A. S. Sandanayake and S. Shinkai, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 2207.
9 R. A. Bissell, A. P. de Silva, H. Q. N. Gunaratne, P. L. M. Lynch, G. E. M. Maguire, C. P. McCoy and K. R. A. S. Sandanayake, *Top. Curr. Chem.*, 1993, **168**, 223.
10 A. P. de Silva, T. Gunnlaugsson and T. E. Rice, *Analyst*, 1996, **121**, 1759.
11 A. W. Czarnik, *Fluorescent Chemosensors for Ion and Molecular Recognition*, American Chemical Society Books, Washington, 1993.
12 A. P. de Silva, H. Q. N. Gunaratne, C. McVeigh, G. E. M. Maguire, P. R. S. Maxwell and E. O'Hanlon, *Chem. Commun.*, 1996, 2191.
13 M. Takeuchi, M. Yamamoto and S. Shinkai, *Chem. Commun.*, 1997, 1731.
14 M. Yamamoto, M. Takeuchi and S. Shinkai, *Tetrahedron*, 1998, **54**, 3125.
15 C. R. Cooper and T. D. James, *Chem. Commun.*, 1997, 1419.
16 A. P. de Silva, H. Q. N. Gunaratne and C. P. McCoy, *Nature*, 1993, **364**, 42.
17 S. Iwata and K. Tanaka, *J. Chem Soc., Chem. Commun.*, 1995, 1491.
18 B. Valeur, J. Pouget, J. Bourson, M. Kaschke and N. P. Ernsting, *J. Phys. Chem.*, 1992, **96**, 6545.
19 C. S. De Ligny and M. Rehbach, *Recl. Trav. Chim. Pays-Bas*, 1960, **79**, 727.
20 R. A. Bissell, E. Calle, A. P. de Silva, S. A. de Silva, H. Q. N. Gunaratne, J. L. Habibjiwan, S. L. A. Peiris, R. A. D. D. Rupasinghe, T. K. S. D. Samarasinghe, K. R. A. S. Sandanayake and J. P. Soumillion, *J. Chem. Soc., Perkin Trans. 2*, 1992, 1559.
21 D. D. Perrin and B. Dempsey, *Buffers for pH and Metal Ion Control*, Chapman and Hall, London, 1974.
22 R. G. Bates, *Determination of pH*, John Wiley & Sons, Inc., London, 1964.
23 S. Feryforgues, M. T. Lebris, J. P. Guette and B. Valeur, *J. Phys. Chem.*, 1988, **92**, 6233.
24 R. M. Izatt, R. E. Terry, B. L. Haymore, L. D. Hansen, N. K. Dalley, A. G. Avondet, J. J. Christensen and B. L. Haymore, *J. Am. Chem. Soc.*, 1976, **98**, 7620.
25 T. T. Hawkins and H. R. Snyder, *J. Am. Chem. Soc.*, 1960, **82**, 3863.
26 Y. Lin, S. A. Lang Jr, C. M. Seiferet, R. G. Child, G. O. Morton and P. F. Fabio, *J. Org. Chem.*, 1979, **44**, 4701.

Paper a909145i