Modular fluorescence sensors for saccharides

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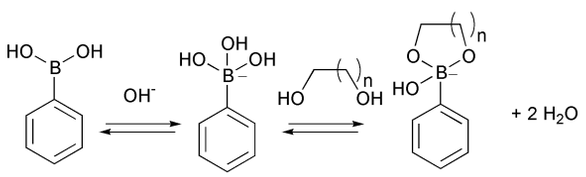
Modular photoinduced electron transfer (PET) sensors bearing two phenylboronic acid groups, a pyrene group and alkylene linkers, from trimethylene to octamethylene, have been prepared and evaluated. The diboronic acid systems with tetramethylene $\mathbf{3}_4$ pentamethylene $\mathbf{3}_5$ and hexamethylene $\mathbf{3}_6$ linkers display the greatest enhancement in binding relative to monoboronic acid **4** with D-glucose. The diboronic acid system with the hexamethylene $\mathbf{3}_6$ linker is particularly D-glucose selective and sensitive. Whilst the diboronic acid systems with the longer heptamethylene $\mathbf{3}_7$ and octamethylene $\mathbf{3}_8$ linkers display the greatest enhancement in binding relative to monoboronic acid **4** with D-galactose. All saccharide titrations were performed in methanolic aqueous solution.

Introduction

A great amount of attention continues to be devoted to the development of synthetic molecular receptors with the ability to recognise neutral organic species, including saccharides.[1,2] The vast majority of these systems have relied upon hydrogen bonding interactions for the purposes of recognition and binding of guest species. However, there is still no designed, monomeric receptor that can compete effectively with bulk water for low concentrations of monosaccharide substrates.[3] As the chemistry of saccharides and related molecular species plays a significant role in the metabolic pathways of living organisms, detecting the presence and concentration of biologically important sugars in aqueous solution is necessary in a variety of medicinal and industrial contexts. The recognition of D-glucose is of particular interest, since the breakdown of D-glucose transport in humans has been correlated with certain diseases: renal glycosuria, cystic fibrosis, diabetes and also human cancer. Recent research provides clear evidence that tight control of blood sugar levels in diabetics sharply reduces the risk of long term complications, which include blindness, kidney failure, heart attacks and gangrene. Industrial applications range from the monitoring of fermentation processes to establishing the enantiomeric purity of synthetic drugs.[4]

The boronic acid–saccharide interaction can be utilised to overcome the problem of undesired solvent–guest competition for the host. Boronic acids readily and reversibly form cyclic boronate esters with diols in aqueous basic media.[4,5] Saccharides contain a linked array of hydroxy groups that provide an ideal structural framework for binding to boronic acids. 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 (Scheme 1).



Scheme 1 Phenylboronic acid complexation with diols.

Because of these properties boronic acid is becoming the receptor of choice in the design of fluorescent sensors for saccharides.[1,2,4–16]

We have been particularly interested in the interaction between boronic acids and amines to create a photoinduced electron transfer (PET) sensory system[17–19] for saccharides.[14,20–22] The interaction of a boronic acid (Lewis acid) and neighbouring tertiary amine (Lewis base) is strengthened on saccharide binding. The strength of this boronic acid–tertiary amine interaction modulates the PET from the amine to the fluorophore. These compounds show increased fluorescence at neutral pH through suppression of the PET from nitrogen to the fluorophore on saccharide binding at neutral pH, a direct result of the stronger boron–nitrogen interaction. Over the last few years we have been interested in developing new fluorescence sensors selective for saccharides employing a modular approach.[14,23,24] The basic idea was to break a sensor into three components; receptor units, linker units, and fluorophore units. The approach requires the selection and synthesis of a set of molecular binding blocks from which the selective fluorescent sensors can be easily constructed. The quick assembly of a diverse selection of fluorescent sensors will require that the receptor and fluorophore units are linked to a core unit using the minimum number of synthetic linkage reactions. The use of common reactions means the synthetic routes towards the new sensors will be convergent. We have previously reported D-glucose sensor **1** with two phenylboronic acid groups (for selectivity) and anthracene (for linker and fluorophore).[21,22] This sensor is the first D-glucose selective PET sensor. We have also reported a PET sensor **2** with two phenylboronic acid groups (for selectivity), two pyrene group (fluorophores) and hexamethylene (for linker).[25] Two fluorophores as with sensor **2** are not required and may in fact be detrimental to an operational sensor since the fluorescence spectra of sensor **2** are complicated by excimer emission due to stacking of the two-pyrene units. The choice of linker is very important because it determines the selectivity for a particular saccharide. We recently communicated our work on the modular PET sensor $\mathbf{3}_6$ with two phenylboronic acid groups (for selectivity), one pyrene group (for fluorophore), and hexamethylene (for linker).[14] Our modular PET sensor $\mathbf{3}_6$ displays high D-glucose selectivity. The modular nature of $\mathbf{3}_6$ means that it is very easy to vary both

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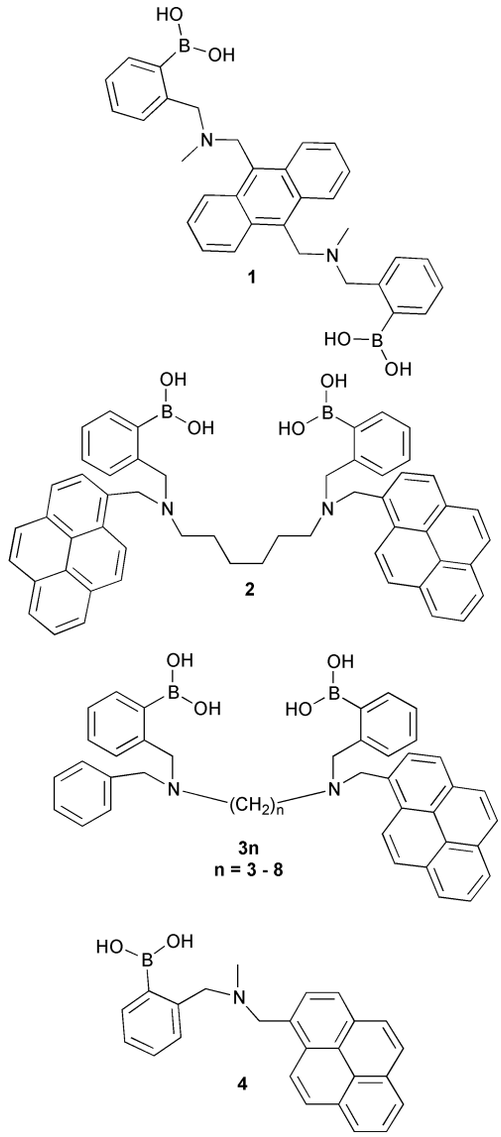
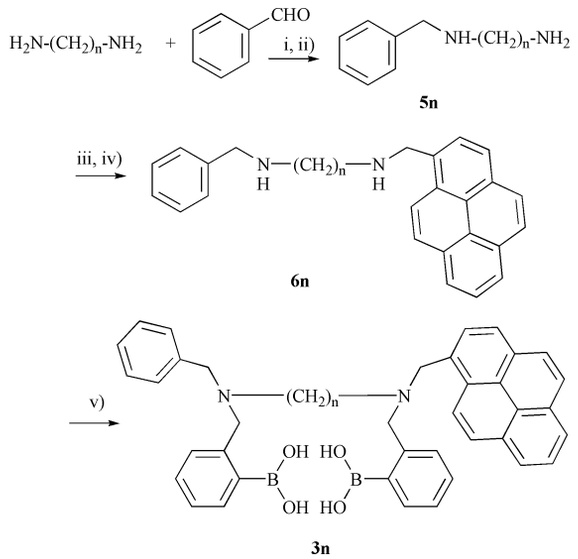


the fluorophore and linker length. Here we report our investigations where we have kept the fluorophore constant (pyrene) and varied the linker **3n** (from trimethylene $n = 3$ to octamethylene $n = 8$). The goal of this research is to reveal the optimum linker length for D-glucose. It is also hoped that the optimum linker length for other monosaccharides will be discovered.

Results and discussion

Syntheses of PET sensors **3n** were achieved according to Scheme 2 from readily available starting materials. Monophenylboronic acid PET sensor **4** was also prepared. The first step is the reaction of alkylenediamine and benzaldehyde in the presence of one equivalent of toluene-*p*-sulfonic acid. When toluene-*p*-sulfonic acid was not used the reaction yield was 18% (hexamethylene), but when one equivalent of toluene-*p*-sulfonic acid was used the reaction yield was increased to 78% (hexamethylene). Work up after reduction by sodium borohydride was achieved by addition of water and extraction using chloroform. The desired compound **5n** dissolves in the chloroform phase, while excess alkylenediamine remained in the water phase (full details are contained in the Experimental section).

Fluorescence titrations of **3n** and **4** (1.0×10^{-7} mol dm^{-3}) with different saccharides were carried out in a pH 8.21 buffer (52.1 wt% methanol in water with KCl, 0.01000 mol dm^{-3}; KH_2PO_4, 0.002752 mol dm^{-3}; Na_2HPO_4, 0.002757 mol dm^{-3}).[26] The fluorescence intensity of **3n** and **4** increased with increasing saccharide concentration (Figs. 1 and 2). The stability constants



Scheme 2 Syntheses of PET sensors **3n**. *Reagents*: i) toluene-*p*-sulfonic acid, THF–EtOH; ii) $NaBH_4$; iii) 1-pyrenecarbaldehyde, THF–MeOH; iv) $NaBH_4$; v) 2-(2-bromobenzyl)-1,3,2-dioxaborinane, K_2CO_3, MeCN.

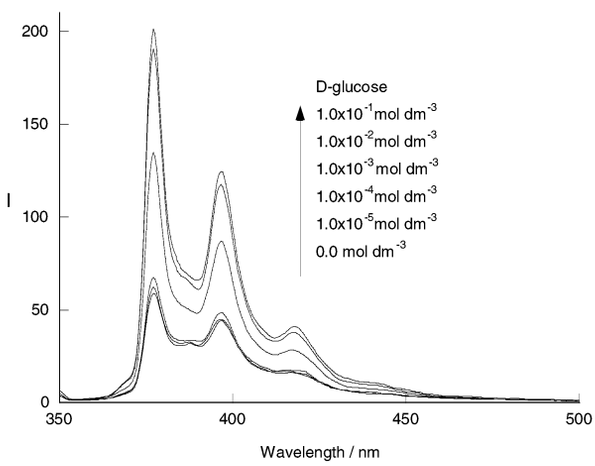


Fig. 1 Fluorescence spectra change of $\mathbf{3}_6$ (1.0×10^{-7} mol dm^{-3}) with different concentrations of D-glucose (0–0.1 mol dm^{-3}) at pH 8.21 in 52.1 wt% methanol. λex 342 nm.

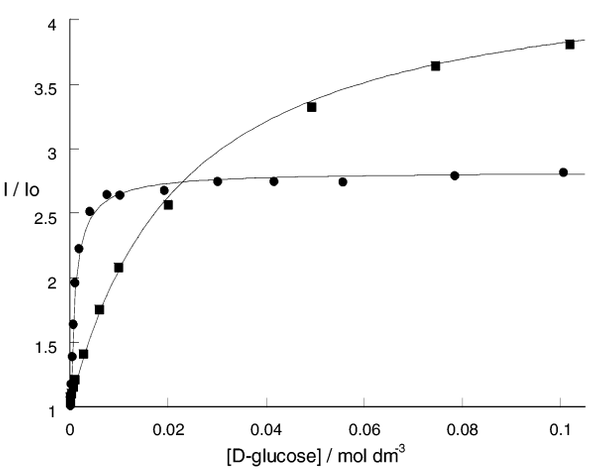


Fig. 2 Plot of relative fluorescence intensity *versus* D-glucose concentration; ● $\mathbf{3}_6$, ■ **4**, $[\mathbf{3}_6] = [\mathbf{4}] = 1.0 \times 10^{-7}$ mol dm^{-3}, at pH 8.21 in 52.1 wt% methanol, [D-glucose] = 0–0.1 mol dm^{-3}, λex at 342 nm, λem at 397 nm.

(K) of PET sensors **3n** and **4** were calculated by fitting the emission wavelength at 397 nm *versus* concentration and are given in Table 1.[26,27]

Table 1 The quantum yield q_{FM} for molecular sensors **3n** and **4** in the absence of saccharides and the stability constant K (determination of coefficient; r^2) and fluorescence enhancement for molecular sensors **3n** and **4** in the presence of saccharides[a]

		D-Glucose		D-Galactose		D-Fructose		D-Mannose	
Sensor	q_{FM}	K/mol^{-1} dm^3	Fluorescence enhancement	K/mol^{-1} dm^3	Fluorescence enhancement	K/mol^{-1} dm^3	Fluorescence enhancement	K/mol^{-1} dm^3	Fluorescence enhancement
$\mathbf{3}_3$	0.16	103 ± 3 (1.00)	3.9	119 ± 5 (1.00)	3.5	95 ± 9 (0.99)	3.6	45 ± 4 (1.00)	2.7
$\mathbf{3}_4$	0.16	295 ± 11 (1.00)	3.3	222 ± 17 (1.00)	3.7	266 ± 28 (0.99)	4.2	39 ± 1 (1.00)	3.4
$\mathbf{3}_5$	0.20	333 ± 27 (1.00)	3.4	177 ± 15 (1.00)	3.0	433 ± 19 (1.00)	3.4	48 ± 2 (1.00)	3.0
$\mathbf{3}_6$	0.24	962 ± 70 (0.99)	2.8	657 ± 39 (1.00)	3.1	784 ± 44 (1.00)	3.2	74 ± 3 (1.00)	2.8
$\mathbf{3}_7$	0.16	336 ± 30 (0.98)	3.0	542 ± 41 (0.99)	2.9	722 ± 37 (1.00)	3.3	70 ± 5 (1.00)	2.7
$\mathbf{3}_8$	0.19	368 ± 21 (1.00)	2.3	562 ± 56 (0.99)	2.3	594 ± 56 (0.99)	2.3	82 ± 3 (1.00)	2.2
4	0.17	44 ± 3 (1.00)	4.5	51 ± 2 (1.00)	4.2	395 ± 11 (1.00)	3.6	36 ± 1 (1.00)	3.7

[a] At pH 8.21 (phosphate buffer) in 52.1 wt% methanol.

The relative stability constants of the diboronic acid **3n** relative to the monoboronic acid **4** are shown in Fig. 3. In most

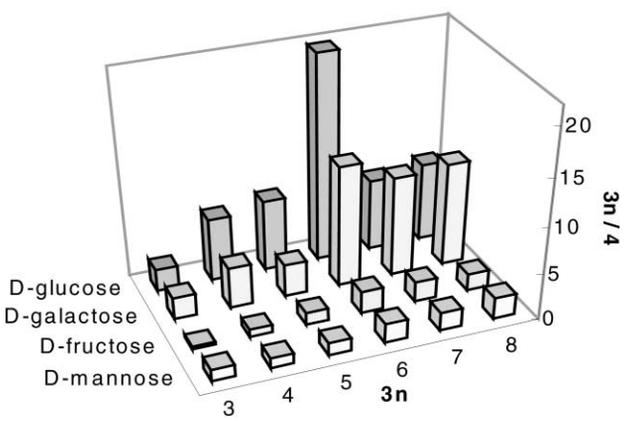


Fig. 3 Relative stability constant K of **3n** *versus* **4** with saccharides.

cases, the stability constants with diboronic acid sensors **3n** are higher than for monoboronic acid sensor **4**. Cooperative binding of the two boronic acid groups is clearly observed as illustrated by the stability constant differences between the mono- and diboronic acid compounds (sensors **4** and **3n** respectively). In particular, the stability constants K for diboronic acid sensors $\mathbf{3}_4$, $\mathbf{3}_5$, $\mathbf{3}_6$, $\mathbf{3}_7$ and $\mathbf{3}_8$ with D-glucose are 6.7, 7.6, 21.9, 7.6 and 8.4 times greater than with monoboronic acid sensor **4**. Whereas the stability constant K of diboronic acid sensors $\mathbf{3}_4$, $\mathbf{3}_5$, $\mathbf{3}_6$, $\mathbf{3}_7$ and $\mathbf{3}_8$ with D-fructose are at most 2 times stronger than monoboronic acid sensor **4**. These results are not surprising since it is well known that D-glucose easily forms 1 : 1 cyclic complexes with diboronic acids, whereas D-fructose tends to form 2 : 1 acyclic complexes with diboronic acids.[1,14,21,25]

Interestingly the stability constant (K) for diboronic acid sensors $\mathbf{3}_7$ and $\mathbf{3}_8$ with D-galactose are 10.6 and 11.0 times stronger than monoboronic acid sensor **4**. This switch in selectivity can be attributed to a larger drop in the D-glucose stability constant (K) than D-galactose stability constant (K) with diboronic acid sensors $\mathbf{3}_7$ and $\mathbf{3}_8$.

The structures of D-glucose and D-galactose are shown in Fig. 4. The 1,2- and 4,6-diols of D-glucose point in the same

Fig. 4 Saccharide structures.

direction (down), but in D-galactose these diols are in opposite directions (1,2-diol is down, 4,6-diol is up). Also, the inter-diol distances of D-glucose are shorter than those of D-galactose. Therefore, it is reasonable to predict that shorter linkers will favour D-glucose and longer linkers will favour D-galactose binding. This is the observed trend as shown in Fig. 3. Sensors $\mathbf{3}_4$, $\mathbf{3}_5$, and $\mathbf{3}_6$, all show higher relative affinity for D-glucose. Whilst sensors $\mathbf{3}_7$, and $\mathbf{3}_8$ display higher relative affinity for D-galactose. What is also evident from Fig. 3 is that a hexamethylene linker ($\mathbf{3}_6$) is the perfect spacer length for D-glucose.

Conclusion

We have shown that it is possible to prepare fluorescence sensors with enhanced D-glucose ($\mathbf{3}_{4-6}$) and D-galactose ($\mathbf{3}_{7-8}$) selectivity using simple building blocks and a modular approach. The PET sensor $\mathbf{3}_6$ which has a hexamethylene linker and two phenylboronic acid groups is both D-glucose selective and sensitive. We believe that these results could be applied in the development of new D-glucose and D-galactose fluorescence sensors. Our ongoing research is directed towards new modular PET sensors with different linker and fluorophore units.

Experimental

General procedures

All chemicals were of commercial reagent quality and were used without further purification with the following exceptions: acetonitrile was distilled with calcium hydride under a nitrogen atmosphere. Gel filtration chromatography was performed using Sephadex LH-20 with methanol as eluent.

^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Bruker AC-300 spectrometer using tetramethylsilane as reference. Mass spectra were obtained on a VG ProSpec spectrometer (Electron Impact, EI) and VG ZabSpec spectrometer (Fast Atomic Bombardment, FAB). Elemental analyses were obtained on a Carlo Erba EA 1110 elemental analyser.

Fluorescence spectra were measured on a Perkin Elmer LS 50B.

N-Benzyl-α,ω-diaminoalkane 5n

A solution of benzaldehyde (1 eq.) in THF (0.2 mol dm^{-3}) was added with heating at reflux to a solution of α,ω-diaminoalkane (5 eq.) and toluene-*p*-sulfonic acid (5 eq.) in ethanol (1 mol dm^{-3}) and then the mixture was heated to reflux for 3 h under a nitrogen atmosphere. After cooling to room temperature, sodium borohydride (3 eq.) was added to the solution which was stirred at room temperature for 1 h. The solvent was removed under reduced pressure and the residue was dissolved in chloroform. The chloroform phase was washed with water, and dried over magnesium sulfate and the solvent was then removed under reduced pressure to yield a yellow oil.

***N*-Benzyl-1,3-diaminopropane $\mathbf{5}_3$.** Yield 209 mg (25%). δ_H (300 MHz, $CDCl_3$, Me_4Si): 1.7 (2H, m, (CH_2)), 2.73 (2H,

t, NHCH_2), 2.79 (2H, t, ArCNCH_2), 3.79 (2H, s, ArCH_2), 7.15–7.25 (5H, m, ArH). δ_C (75 MHz, $CDCl_3$, Me_4Si): 33.6, 40.5, 47.2, 54.0, 126.6, 127.8, 128.1, 140.1.

***N*-Benzyl-1,4-diaminobutane 5$_4$.** Yield 316 mg (35%). δ_H (300 MHz, $CDCl_3$, Me_4Si): 1.1–1.5 (4H, m, (CH_2)$_2$), 2.65 (2H, t, NHCH_2), 2.71 (2H, t, ArCNCH_2), 3.78 (2H, s, ArCH_2), 7.15–7.25 (5H, m, ArH). δ_C (75 MHz, $CDCl_3$, Me_4Si): 28.0, 31.6, 42.2, 49.3, 54.1, 126.7, 127.9, 128.2, 140.3.

***N*-Benzyl-1,5-diaminopentane 5$_5$.** Yield 183 mg (19%). δ_H (300 MHz, $CDCl_3$, Me_4Si): 1.36–1.61 (6H, m, (CH_2)$_3$), 2.64 (2H, t, NHCH_2), 2.66 (2H, t, ArCNCH_2), 3.79 (2H, s, ArCH_2), 7.15–7.25 (5H, m, ArH). δ_C (75 MHz, $CDCl_3$, Me_4Si): 24.6, 30.0, 33.7, 42.2, 49.4, 54.1, 126.8, 128.1, 128.3, 140.9.

***N*-Benzyl-1,6-diaminohexane 5$_6$.** Yield 1.61 g (78%). δ_H (300 MHz, $CDCl_3$, Me_4Si): 1.1–1.5 (8H, m, (CH_2)$_4$), 2.55 (2H, t, NHCH_2), 2.65 (2H, t, ArCNCH_2), 3.75 (2H, s, ArCH_2), 7.15–7.25 (5H, m, ArH). δ_C (75 MHz, $CDCl_3$, Me_4Si): 26.9, 27.3, 30.2, 33.8, 42.2, 49.4, 54.1, 126.7, 127.9, 128.2, 140.4.

***N*-Benzyl-1,7-diaminoheptane 5$_7$.** Yield 196 mg (49%). δ_H (300 MHz, $CDCl_3$, Me_4Si): 1.36–1.58 (6H, m, (CH_2)$_3$), 2.64 (2H, t, NHCH_2), 2.66 (2H, t, ArCNCH_2), 3.79 (2H, s, ArCH_2), 7.15–7.25 (5H, m, ArH). δ_C (75 MHz, $CDCl_3$, Me_4Si): 24.6, 30.0, 33.7, 42.2, 49.4, 54.1, 126.8, 128.1, 128.3, 140.9.

***N*-Benzyl-1,8-diaminooctane 5$_8$.** Yield 542 mg (46%). δ_H (300 MHz, $CDCl_3$, Me_4Si): 1.3–1.5 (12H, m, (CH_2)$_6$), 2.62 (2H, t, NHCH_2), 2.66 (2H, t, ArCNCH_2), 3.78 (2H, s, ArCH_2), 7.2–7.3 (5H, m, ArH). δ_C (75 MHz, $CDCl_3$, Me_4Si): 27.2, 27.7, 29.8, 29.9, 30.5, 34.1, 42.5, 49.8, 54.4, 127.0, 128.3, 128.5, 140.7.

N-Benzyl-*N′*-pyren-1-ylmethyl-α,ω-diaminoalkane 6n

A solution of **5n** (1 eq.) and 1-pyrenecarbaldehyde (1.1 eq.) in THF and methanol (0.1 mol dm^{-3}, 50 : 50 vol%) was stirred at room temperature for 7 h under a nitrogen atmosphere. Sodium borohydride (3.3 eq.) was added to the solution which was stirred at room temperature for 1 h. The solvent was removed under reduced pressure and the residue was dissolved in chloroform. The chloroform phase was washed with water, dried over magnesium sulfate and the solvent removed under reduced pressure. The residue was purified by gel filtration chromatography to yield a yellow oil.

***N*-Benzyl-*N′*-pyren-1-ylmethyl-1,3-diaminopropane 6$_3$.** Yield 417 mg (55%). *m*/*z* (EI) 379 ([M]$^+$, 100%); Found: C, 85.08; H, 6.78; N, 7.35. $C_{27}H_{26}N_2$ +0.07 CH_3OH requires C, 85.31; H, 6.97; N, 7.36%; δ_H (300 MHz, $CDCl_3$, Me_4Si): 1.78 (2H, m, CH_2), 2.73 (2H, t, BnNCH_2), 2.86 (2H, t, PyCNCH_2), 3.79 (2H, s, PhCH_2), 4.49 (2H, s, PyCH_2), 7.15–7.25 (5H, m, Ph–H), 7.97–8.18, 8.37 (8H, 1H respectively, m, d, Py–H). δ_C (75 MHz, $CDCl_3$, Me_4Si): 30.2, 48.0, 48.5, 51.9, 54.0, 123.1, 124.5, 124.8, 124.9, 125.7, 126.7, 126.8, 126.9, 127.3, 127.4, 128.0, 128.2, 128.9, 129.2, 130.5, 130.7, 131.2, 133.9, 140.3.

***N*-Benzyl-*N′*-pyren-1-ylmethyl-1,4-diaminobutane 6$_4$.** Yield 467 mg (60%). *m*/*z* (EI) 392 ([M]$^+$, 27%); Found: C, 85.18; H, 7.41; N, 7.17. $C_{28}H_{28}N_2$ + 0.07 CH_3OH requires C, 85.39; H, 7.23; N, 7.10%; δ_H (300 MHz, $CDCl_3$, Me_4Si): 1.51 (4H, m, (CH_2)$_2$), 2.65 (2H, t, BnNCH_2), 2.78 (2H, t, PyCNCH_2), 3.76 (2H, s, PhCH_2), 4.48 (2H, s, PyCH_2), 7.15–7.25 (5H, m, Ph–H), 7.93–8.22, 8.37 (8H, 1H respectively, m, d, Py–H). δ_C (75 MHz, $CDCl_3$, Me_4Si): 28.0, 49.3, 49.9, 51.9, 54.1, 123.2, 124.7, 125.1, 125.9, 127.1, 127.5, 127.7, 128.1, 128.4, 131.4.

***N*-Benzyl-*N′*-pyren-1-ylmethyl-1,5-diaminopentane 6$_5$.** Yield 384 mg (47%). *m*/*z* (EI) 406 ([M]$^+$, 12%); δ_H (300 MHz, $CDCl_3$, Me_4Si): 1.40 (2H, m, CH_2), 1.58 (4H, m, NCCH_2), 2.63 (2H, t, BnNCH_2), 2.80 (2H, t, PyCNCH_2), 3.78 (2H, s, PhCH_2), 4.49 (2H, s, PyCH_2), 7.15–7.28 (5H, m, Ph–H), 7.97–8.07, 8.09–8.19, 8.37 (4H, 4H, 1H respectively, m, m, d, Py–H). δ_C (75 MHz, $CDCl_3$, Me_4Si): 25.3, 30.2, 49.6, 50.1, 52.1, 54.3, 123.3, 124.8, 125.1, 125.2, 126.0, 127.0, 127.1, 127.6, 127.8, 128.3, 128.5, 130.7, 131.0, 131.5, 134.3, 140.7.

***N*-Benzyl-*N′*-pyren-1-ylmethylhexane-1,6-diamine 6$_6$.** Yield 386 mg (86%). *m*/*z* (EI) 420 ([M]$^+$, 7%); δ_H (300 MHz, $CDCl_3$, Me_4Si): 1.32 (4H, m, (CH_2)$_2$), 1.45 (2H, m, BnNCCH_2), 1.55 (2H, m, PyCNCCH_2), 2.55 (2H, t, BnNCH_2), 2.75 (2H, t, PyCNCH_2), 3.75 (2H, s, PhCH_2), 4.50 (2H, s, PyCH_2), 7.15–7.25 (5H, m, Ph–H), 7.95–8.10, 8.15–8.22, 8.37 (4H, 4H, 1H respectively, m, m, d, Py–H). δ_C (75 MHz, $CDCl_3$, Me_4Si): 27.4, 30.2, 49.5, 50.0, 51.9, 54.1, 54.2, 123.2, 124.7, 125.0, 125.1, 125.9, 126.9, 127.0, 127.5, 127.6, 128.1, 128.4, 129.1, 131.3, 134.2.

***N*-Benzyl-*N′*-pyrene-1-ylmethyl-1,7-diaminoheptane 6$_7$.** Yield 420 mg (48%). *m*/*z* (EI) 434 ([M]$^+$, 12%); Found: C, 85.61; H, 8.04; N, 6.38. $C_{31}H_{34}N_2$ requires C, 85.65; H, 7.90; N, 6.45%; δ_H (300 MHz, $CDCl_3$, Me_4Si): 1.30–1.58 (10H, m, (CH_2)$_5$), 2.58 (2H, t, BnNCH_2), 2.79 (2H, t, PyCNCH_2), 3.78 (2H, s, PhCH_2), 4.50 (2H, s, PyCH_2), 7.21–7.33 (5H, m, Ph–H), 8.01–8.21 and 8.38 (8H, 1H respectively, m, d, Py–H). δ_C (75 MHz, $CDCl_3$, Me_4Si): 27.4, 29.6, 30.2, 49.5, 50.1, 52.0, 54.2, 68.1, 123.2, 124.7, 125.0, 125.1, 125.9, 126.9, 127.0, 127.5, 127.6, 128.1, 128.4, 130.6, 130.9, 131.3, 134.2, 140.6.

***N*-Benzyl-*N′*-pyrene-1-ylmethyl-1,8-diaminooctane 6$_8$.** Yield 641 mg (67%). *m*/*z* (EI) 448 ([M]$^+$, 34%); Found: C, 85.58; H, 8.19; N, 6.33. $C_{32}H_{36}N_2$ requires C, 85.65; H, 8.10; N, 6.24%; δ_H (300 MHz, $CDCl_3$, Me_4Si): 1.3–1.6 (12H, m, (CH_2)$_6$), 2.63 (2H, t, BnNCH_2), 2.79 (2H, t, PyCNCH_2), 3.79 (2H, s, PhCH_2), 4.50 (2H, s, PyCH_2), 7.25–7.32 (5H, m, Ph–H), 7.98–8.21, 8.48 (8H, 1H respectively, m, d, Py–H). δ_C (75 MHz, $CDCl_3$, Me_4Si): 27.4, 29.5, 30.2, 49.6, 50.1, 52.0, 54.2, 123.2, 124.7, 125.0, 125.9, 126.9, 127.0, 127.5, 128.1, 128.4, 134.2.

N-Benzyl-*N,N′*-bis(2-dihydroxyborylbenzyl)-*N′*-pyren-1-ylmethyl-α,ω-diaminoalkane 3n

A solution of **6n** (1 eq.), potassium carbonate (4 eq.), and 2-(2-bromobenzyl)-1,3,2-dioxaborinane (2.4 eq.) in acetonitrile (0.05 mol dm^{-3}) was heated at reflux for 7 h under a nitrogen atmosphere. The solvent was removed under reduced pressure, and the residue was dissolved in chloroform. The chloroform phase was washed with water and dried over magnesium sulfate, and the solvent was removed under reduced pressure. The residue was reprecipitated from chloroform and *n*-hexane to yield a yellow powder.

***N*-Benzyl-*N,N′*-bis(2-dihydroxyborylbenzyl)-*N′*-pyren-1-ylmethyl-1,3-diaminopropane 3$_3$.** Yield 221 mg (83%). Mp 165 °C (decomposed); *m*/*z* (FAB) 1187 ([M + H + 4(3 − $HOCH_2C_6H_4NO_2$) − 4(H_2O)]$^+$, 100%); Found: C, 77.31; H, 6.39; N, 4.37. $C_{41}H_{42}B_2N_2O_4$–H_2O + 0.04 $CHCl_3$ requires C, 77.59; H, 6.37; N, 4.41%; δ_H (300 MHz, $CDCl_3$ + CD_3OD (a few drops), Me_4Si): 1.65 (2H, m, CH_2), 2.07 (2H, t, BnNCH_2), 2.24 (2H, t, PyCNCH_2), 3.39 (2H, s, PhB(OH)CH_2NBn), 3.48 (2H, s, PhB(OH)CH_2NCPy), 3.79 (2H, s, PhCH_2), 4.17 (2H, s, PyCH_2), 6.86–7.32 and 7.68–8.13 (22H, m, m, Ar–H). δ_C (75 MHz, $CDCl_3$ + CD_3OD (a few drops), Me_4Si): 20.4, 51.6, 52.9, 54.4, 57.1, 61.2, 62.0, 123.4, 125.0, 125.5, 125.7, 126.4, 127.3, 127.4, 127.7, 127.8, 128.8, 129.0, 129.8, 130.4, 131.1, 131.3, 131.6, 136.4, 141.5, 141.6.

***N*-Benzyl-*N,N′*-bis(2-dihydroxyborylbenzyl)-*N′*-pyren-1-ylmethyl-1,4-diaminobutane 3$_4$.** Yield 239 mg (56%). Mp 147–151 °C; *m*/*z* (FAB) 1201 ([M + H + 4(3 − $HOCH_2C_6H_4NO_2$) −

4(H_2O)]$^+$, 100%); Found: C, 76.75; H, 6.63; N, 4.27. $C_{42}H_{42}B_2N_2O_4$ + 0.1 C_6H_{14} requires C, 76.46; H, 6.55; N, 4.18%; δ_H (300 MHz, $CDCl_3$ + CD_3OD (a few drops), Me_4Si): 1.15–1.31 (4H, m, (CH_2)$_2$)), 2.17 (2H, t, BnNCH_2), 2.38 (2H, t, PyCNCH_2), 3.29 (2H, s, PhB(OH)CH_2NBn), 3.48 (2H, s, PhB(OH)CH_2NCPy), 3.80 (2H, s, PhCH_2), 4.14 (2H, s, PyCH_2), 6.90–7.31 (13H, m, Ar–*H*), 7.70–8.12 (9H, m, Py–*H*). δ_C (75 MHz, $CDCl_3$ + CD_3OD (a few drops), Me_4Si): 22.8, 23.0, 51.6, 52.9, 54.4, 57.1, 61.2, 62.0, 123.2, 124.7, 125.2, 125.3, 126.0, 127.3, 127.4, 127.48, 127.53, 127.6, 128.4, 128.7, 129.5, 129.9, 131.26, 131.31, 136.1, 141.5, 141.6.

***N*-Benzyl-*N*,*N*′-bis(2-dihydroxyborylbenzyl)-*N*′-pyren-1-ylmethyl-1,5-diaminopentane 3_5.** Yield 241 mg (72%). Mp 162–166 °C; *m*/*z* (FAB) 1215 ([M + H + 4(3 − $HOCH_2C_6H_4NO_2$) − 4(H_2O)]$^+$, 100%); Found: C, 76.60; H, 6.94; N, 3.98. $C_{43}H_{44}B_2N_2O_4$ + 0.05 C_6H_{14} requires C, 76.61; H, 6.65; N, 4.13%; δ_H (300 MHz, $CDCl_3$ + CD_3OD (a few drops), Me_4Si): 1.28 (2H, m, CH_2), 1.42 (4H, m, NCCH_2), 2.19 (2H, t, BnNCH_2), 3.37 (2H, s, PhB(OH)CH_2NBn), 3.52 (2H, s, PhB(OH)CH_2NCPy), 3.73 (2H, s, PhCH_2), 4.21 (2H, s, PyCH_2), 7.02–7.39 and 7.89–8.19 (22H, m, Ar–*H*), 8.25 (4H, br s, BO*H*). δ_C (75 MHz, $CDCl_3$ + CD_3OD (a few drops), Me_4Si): 25.7, 27.2, 30.1, 32.7, 49.9, 51.9, 62.8, 123.1, 124.7, 125.0, 125.1, 125.9, 127.1, 127.5, 127.7, 129.0, 130.7, 130.9, 131.3, 134.0.

***N*-Benzyl-*N*,*N*′-bis(2-dihydroxyborylbenzyl)-*N*′-pyren-1-ylmethylhexane-1,6-diamine 3_6.** Yield 172 mg (35%). Mp 165–168 °C; *m*/*z* (FAB) 1230 ([M + H + 4(3 − $HOCH_2C_6H_4NO_2$) − 4(H_2O)]$^+$, 100%); Found: C, 76.56; H, 6.69; N, 3.80. $C_{44}H_{46}B_2N_2O_4$ requires C, 76.76; H, 6.73; N, 4.07%; δ_H (300 MHz, $CDCl_3$ + CD_3OD (a few drops), Me_4Si): 1.25–1.48 (8H, m, (CH_2)$_4$), 2.23 (2H, t, BnNCH_2), 2.50 (2H, t, PyCNCH_2), 3.50 (2H, s, PhB(OH)CH_2NBn), 3.63 (2H, s, PhB(OH)CH_2NCPy), 3.90 (2H, s, PhCH_2), 4.21 (2H, s, PyCH_2), 7.02–7.41 and 7.88–8.19 (22H, m, Ar–*H*); δ_C (75 MHz, $CDCl_3$ + CD_3OD (a few drops), Me_4Si): 24.4, 24.8, 26.9, 27.1, 51.8, 53.2, 54.2, 57.1, 61.2, 62.0, 123.2, 124.7, 125.1, 125.3, 126.0, 127.3, 127.4, 127.5, 127.6, 128.4, 128.6, 129.6, 129.9, 130.1, 131.25, 131.32, 136.1, 141.6, 141.8.

***N*-Benzyl-*N*,*N*′-bis(2-dihydroxyborylbenzyl)-*N*′-pyren-1-ylmethyl-1,7-diaminoheptane 3_7.** Yield 185 mg (57%). Mp 158–163 °C; *m*/*z* (FAB) 1243 ([M + H + 4(3 − $HOCH_2C_6H_4NO_2$) − 4(H_2O)]$^+$, 100%); Found: C, 75.12; H, 7.01; N, 3.70. $C_{44}H_{46}B_2N_2O_4$ + 0.15 $CHCl_3$ requires C, 75.26; H, 6.75; N, 3.89%; δ_H (300 MHz, $CDCl_3$ + CD_3OD (a few drops), Me_4Si): 1.20–1.47 (10H, m, (CH_2)$_5$), 2.20 (2H, t, BnNCH_2), 2.46 (2H, t, PyCNCH_2), 3.42 (2H, s, PhB(OH)CH_2NBn), 3.59 (2H, s, PhB(OH)CH_2NCPy), 3.83 (2H, s, PhCH_2), 4.21 (2H, s, PyCH_2), 7.02–7.30 and 7.78–8.12 (22H, m, Ar–*H*). δ_C (75 MHz, $CDCl_3$ + CD_3OD (a few drops), Me_4Si): 24.8, 25.1, 27.4, 27.6, 29.2, 52.2, 53.5, 54.5, 57.4, 61.6, 62.3, 123.5, 125.0, 125.4, 125.6, 126.3, 127.6, 127.8, 127.9, 128.8, 128.9, 129.9, 130.3, 130.4, 131.2, 131.3, 131.6, 136.5, 142.0, 142.1.

***N*-Benzyl-*N*,*N*′-bis(2-dihydroxyborylbenzyl)-*N*′-pyren-1-ylmethyl-1,8-diaminooctane 3_8.** Yield 248 mg (63%). Mp 121–125 °C; *m*/*z* (FAB) 1257 ([M + H + 4(3 − $HOCH_2C_6H_4NO_2$) − 4(H_2O)]$^+$, 100%); Found: C, 77.04; H, 7.15; N, 4.03. $C_{46}H_{50}B_2N_2O_4$ requires C, 77.09; H, 7.05; N, 3.91%; δ_H (300 MHz, $CDCl_3$ + CD_3OD (a few drops), Me_4Si): 1.3–1.7 (12H, m, (CH_2)$_6$), 2.25 (2H, t, BnNCH_2), 2.46 (2H, t, PyCNCH_2), 3.52 (2H, s, PhB(OH)CH_2NBn), 3.67 (2H, s, PhB(OH)CH_2NCPy), 3.88 (2H, s, PhCH_2), 4.22 (2H, s, PyCH_2), 7.11–7.35 and 7.85–8.21 (22H, m, Ar–*H*). δ_C (75 MHz, $CDCl_3$ + CD_3OD (a few drops), Me_4Si): 23.0, 25.1, 27.5, 27.6, 29.6, 32.0, 52.3, 53.5, 54.5, 57.4, 61.5, 62.3, 125.0, 125.4, 125.6, 126.3, 127.6, 127.8, 127.9, 128.75, 128.83, 129.0, 129.9, 130.2, 130.3, 130.4, 131.2, 131.6, 136.5, 142.0, 142.1.

Fluorescence-saccharide titrations of 3n and 4 at pH 8.21

The fluorescence spectra of **3n** and **4** (1.0×10^{-7} mol dm^{-3}) in a pH 8.21 buffer (52.1 wt% methanol in water with KCl, 0.01000 mol dm^{-3}; KH_2PO_4, 0.002752 mol dm^{-3}; Na_2HPO_4, 0.002757 mol dm^{-3})[26] were recorded as increasing amounts of saccharide were added to the solution.

Measurement of quantum yield

A solution of pyrene in absolute ethanol adjusted to an absorbance of 0.042 at 334 nm was degassed using nitrogen. The fluorescence spectrum of this solution excited at 334 nm was recorded from 350 nm to 550 nm. Solutions of sensors $\mathbf{3_3}$, $\mathbf{3_4}$, $\mathbf{3_5}$, $\mathbf{3_6}$, $\mathbf{3_7}$, $\mathbf{3_8}$ and **4** in 52.1 wt% methanol at pH 8.21 were adjusted to an absorbance of 0.054, 0.058, 0.059, 0.056, 0.055, 0.059, and 0.057, respectively, at 334 nm. The fluorescence spectra of these sensors excited at 334 nm were also recorded from 350 nm to 550 nm. The areas under the fluorescence spectra were calculated using KaleidaGraph version 3.51 for PC, published by Synergy Software and developed by Abelbeck Software, 2457 Perikiomen Avenue, Reading, PA 19606. The quantum yield of **3n** and **4** were then calculated by comparison with pyrene as standard using the following equation:[28]

$$q_s = q_{Py} \times (FA_s/FA_{Py}) \times (A_{Py}/A_s)$$

q is quantum yield; FA is the area under the fluorescence spectra; A is the absorbance at 334 nm; $_s$ and $_{Py}$ are sensors (**3n** and **4**) and the pyrene reference, respectively. 0.72 is used as the reference quantum yield of pyrene in ethanol.[29]

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