A New Glucose-Selective Fluorescent Bisboronic Acid. First Report of Strong α-Furanose Complexation in Aqueous Solution at Physiological pH[1]

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A new bisboronic acid based glucose sensor **7** was synthesized from 9,10-bis(chloromethyl)anthracene and 2,2-dimethyl-1,3-propanediol protected 3-pyridineboronic acid. Due to its ionic structure **7** was found sufficiently water soluble for carbohydrate binding studies at neutral pH by means of NMR spectroscopy, fluorometry, and potentiometry. The pK_a's of **7** have been determined to 3.7 and 4.7 by potentiometric titration. From a solution of **7** and glucose (1:1) in water (pH 7.4) we observed the formation of a bisdentate boronic acid complex **7·Glu** which has been assigned to a 1,2:3,5 bound α-D-glucofuranose complex. The evidence for this furanose structure comprises ^{1}H and ^{13}C NMR data with emphasis on the information from $^1J_{C-C}$ coupling constants. Complex **7·Glu** shows an increased fluorescence compared to **7**. The stability constant for the 1:1 complex (log K = 3.4) was determined from fluorometric titration, potentiometry, and NMR spectroscopy. Boronic acid **7** shows good selectivity for glucose compared to fructose and galactose.

Introduction

There is today a strong demand for the development of new, efficient, selective, and cheap glucose sensors for, for example, diabetes therapy.[2,3] Lately aromatic boronic acids have gained considerable attention as a new and very promising alternative to the well-established enzyme-based technology which tend to be complex and expensive.[4] Boronic acids are capable of reversible formation of strong covalent bonds with the diol functionalities of carbohydrates in form of cyclic esters[5−7] and are, with respect to binding in aqueous solution, superior to other sensor systems involving weaker noncovalent or hydrogen bonded interactions.[8] To be able to further enhance binding and especially to enable discrimination between various carbohydrates, the concept of having a bisdentate binding of carbohydrates by two boronic acid moieties (Figure 1) was suggested.[9,10]

Shinkai's group has continued exploring this concept and has synthesized new sensor molecules now reported to be capable of distinction between various carbohydrates[11] and in one case even between enantiomeric compounds.[12] For examination of the recognition events sensoric methods which cover a broad range of techniques such as fluorescence,[13−15] UV−vis absorption,[16,17] circular dichroism,[10,18] and electrochemistry[19] have been used.

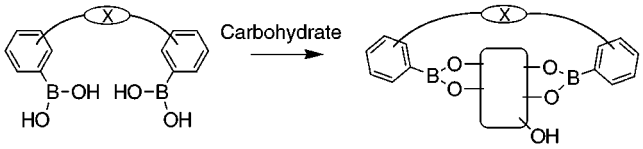

Figure 1. The concept of bisdentate binding of carbohydrates by aromatic boronic acids.

The ester formation between boronic acids and diols in aqueous solution is strongly pH dependent according to the equilibria depicted in Figure 2. For glucose measurements in for example, whole blood, complex formation at nearly neutral pH is essential. To obtain a favorable binding, the hydroxyboronate **D** (Figure 2), containing a tetrahedral boron, is to be considered and as pK_a(2) is 1−2 units lower than pK_a(1)[20,21] this complex

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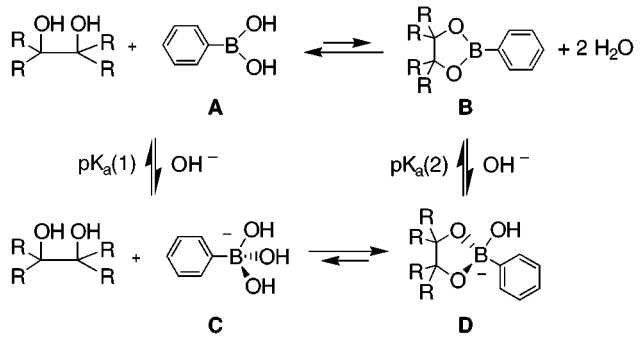

Figure 2. Equilibria between boronic acids and diols.

will only exist in fair amounts at neutral pH if the boronic acid itself has a p$K_a \leq 7$. Fulfilling this demand for the pK_a value of the boronic acids is in fact the major problem for most of the suggested boronic acid based sensors, as in these derivatives the aromatic boronic acids have pK_a values in a range from 8 to 10 and therefore require more or less alkaline conditions to form strong complexes.[22] A lowering of this value can be obtained by having strong electron-withdrawing substituents on the aromatic moiety as, for example, in 4-carboxy-3-nitrophenylboronic acid[23] which has a pK_a of 7.0,[24] but this is not a simple modification considering the chemistry of boronic acids.[25] Another very attractive modification of the boronic acids is the incorporation of an *o*-aminomethyl substituent. As originally shown by G. Wulff and co-workers[26,27] this arrangement, due to a favorable B−N interaction, substantially lowers the pK value for the equilibrium leading to a complexing tetragonal boron to, for example, a pK value of 5.2 for *o*-[(*N*,*N*-dimethylamino)methyl]phenylboronic acid. This latter concept has recently been applied in an elegant way by Shinkai and co-workers in the design of a glucose-selective fluorescent anthracene based sensor.[28,29] This sensor molecule, which is insoluble in water, was reported to form a pyranose complex with glucose.[30]

Boronic acids are known to bind glucose preferentially in the α-furanose form and not in the more aboundant α-pyranose form,[22,31,32] but the binding sites around hydroxy groups 3, 5, and 6 may vary.

As we find knowledge of the precise complex structures essential for the development of new custom-made sensors, we designed a new bisboronic acid based glucose sensor which could fulfill the requirements of (1) low pK_a's of the boronic acids for binding studies at neutral pH, (2) an acceptable water solubility to allow for structure determination of the complex in water by NMR methods, and (3) a fluorophoric group for sensory output. We chose 3-pyridineboronic acid as the anchor, as it, due to the formation of a favored zwitterionic pyridinium hydroxyboronate, has a *very* low pK_a value of 4.0.[33,34] Furthermore alkylation of the nitrogen would give a pyridinium salt with an expected enhanced water solubility. We decided after some model considerations on a design of a bisboronic acid comprising two 3-pyridineboronic acids grafted onto an anthracene moiety.

Results and Discussion

The bisboronic acid derivative **6** was synthesized according to Scheme 1. The initial protected product **4** was deprotected by stirring in acetone−water (10:1). This gave the crude free boronic acid **5** which was purified by crystallization and recrystallization from MeOH to give the 1,3-dimethyldiboroxane **6** in 72% overall yield. The structure of the intermediate crude boronic acid **5** was deduced from elemental analysis as well as IR and NMR spectroscopy. We found that **5** contained 1 equiv of acetone as the IR spectrum in KBr unveiled a C=O absorption at 1706 cm^{-1}, and the ^1H NMR spectrum in CD_3OD showed peaks at 1.29 and 2.15 ppm which integrated to a total of 6H. Upon addition of one drop of D_2O the signal at 1.29 ppm practically disappeared and the one at 2.15 ppm increased. This observation is explained by the fact that the boronic acid catalyzes an equilibrium between acetone, methanol-d_4, and 2,2-dimethoxy-d_6-propane. This was verified by the addition of one drop of 2,2-dimethoxypropane which, in addition to the OMe/MeOH peak at 3.34 ppm, resulted in an increase of both the 1.29 ppm and the acetone signal.

The structure of the diboroxane **6** was established on the basis of electron spray mass spectrometry, elemental analysis, and NMR spectroscopy. The ESMS spectrum of a MeOH solution of **6** showed *m*/*z* 491 corresponding to the M^+ ion of **6**+OMe. Furthermore the M^{2+} of the bisboronic acid **7** and its mono- and dimethyl ester could be observed whereas no evidence for the tri- nor tetramethyl ester were obtained. ^1H NMR in CD_3OD showed a signal at 3.34 ppm integrating to 9H. This integral was accounted for by crystallization of **6** with 1 equiv of MeOH in agreement with the elemental analysis. Upon dissolvation in water, diboroxane **6** hydrolyzes fast into the bisboronic acid **7**. Considering earlier work of Fisher and Havinga[33] who have shown some hydrolytical instability of certain pyridineboronic acids, we observed no such instability of **7** at room temperature. The ^1H NMR spectrum of **7** in D_2O (pH ∼ 3 upon dissolution) showed broad lines, and at a certain concentration a gel was formed, suggesting the presence of polymeric species.

Glucose Complexation. Due to our earlier investigation on a bisboronic acid[22] and a simple model study, we anticipated a 1:1 complex between **7** and glucose and thus a 1:1 solution of **6** and D-glucose in D_2O (pD = 7.4) was prepared (see Experimental Section). The ^1H and ^{13}C NMR spectra of this mixture (sharp lines) unveiled the formation of a complex named **7·Glu** (∼95%) in admixture with free **7** (∼5%) and free glucose (∼5%). The assigned ^{13}C and ^1H chemical shifts of **7·Glu** are given in Tables 1 and 2, respectively. The measured $^3J_{H-H}$

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Scheme 1

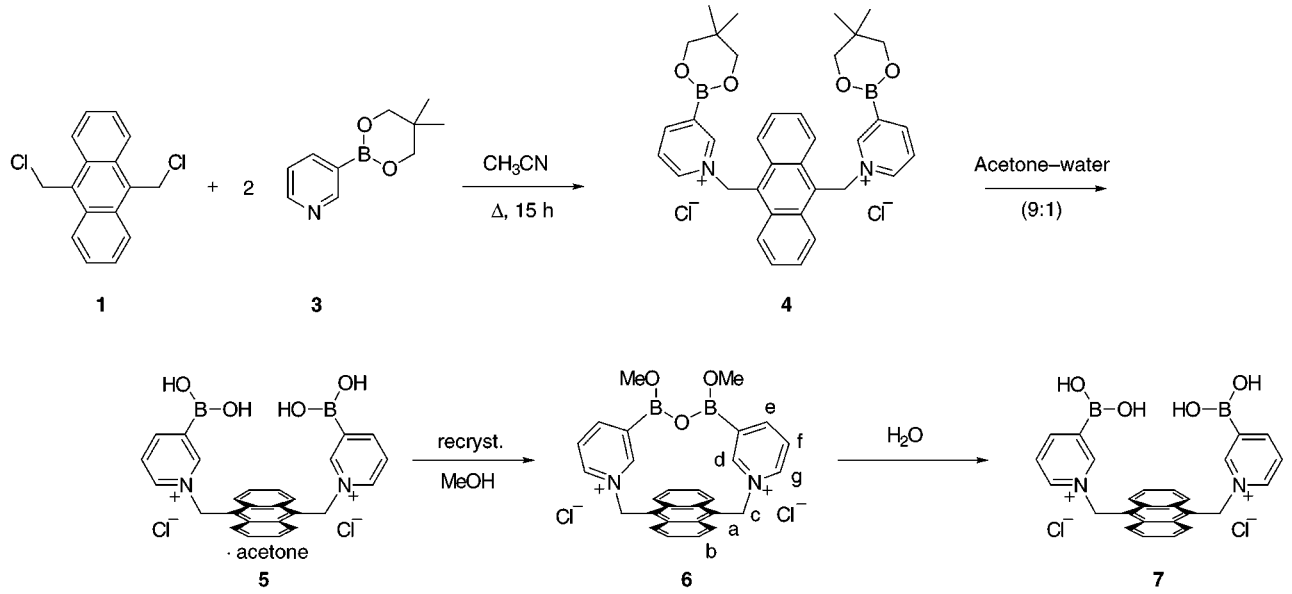

Table 1. ^{13}C Chemical Shifts (ppm) and $^1J_{CC}$ Coupling Constants (Hz) for the Glucose Part of Boronic Acid Complexes and Model Compounds

compound	C-1	C-2	C-3	C-4	C-5	C-6	J_{C1-C2}	J_{C2-C3}	J_{C3-C4}	J_{C4-C5}	J_{C5-C6}
7·Glu[a]	106.6	84.7	79.3	82.0	73.0	67.0	35.9	43.5	36.6	43.5	43.5
7·Glu$_{6d}$[a]	106.5	85.0	78.9	87.0	68.0	22.6					
8[e]	107.9	87.7	76.6	84.1	75.6	69.2	34.0	42.3	38.2	48.1	34.2
9[b]	104.3	83.4	77.0	72.3	74.3	60.7	33.5	46.4	34.6	41.6	40.6
10[b]	104.0	85.8	73.6	74.7	70.8	62.0	34.4	44.0	34.4	40.5	40.9
11[c]	107.6	85.7	79.5	81.1	73.9	67.0	35.7	43.6	34.3	40.0	34.5
α-D-Glucopyranose[d]	92.7	72.1	73.3	70.4	72.1	61.3	45.2	37.8	37.3	40.3	42.7
β-D-Glucopyranose[d]	96.5	74.8	76.4	70.3	76.6	61.5	45.2	38.8	39.1	40.7	43.3

[a] In D_2O at pD = 7.4. [b] In DMSO-d_6. [c] In D_2O at pD = 10−11. [d] In D_2O. [e] Chemical shifts given for D_2O solution, coupling constants for a DMSO-d_6 solution.

Table 2. ^{1}H Chemical Shifts (ppm) for the Glucose Part of Boronic Acid Complexes and Reference Compounds

compound	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
7·Glu[a]	5.52	3.73	1.67	1.69	3.10	2.85	2.62
7·Glu$_{6d}$[a]	5.48	3.71	1.70	1.53	3.08	0.50	−
11[b,c]	6.01	4.40	4.22	3.94	4.44	3.73	3.68
12[b]	5.77	4.43	4.09	3.71	4.02	3.82	3.42

[a] In D_2O at pD = 7.4. [b] In D_2O at pD = 11. [c] At −1 °C.

Table 3. J_{H-H} Coupling Constants (Hz) for the Glucose Part of Boronic Acid Complexes and Model Compounds

compound	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6a}$	$J_{5,6b}$	$J_{6a,6b}$
7·Glu[a]	3.7	∼0	4.4	7.3	7.5	3.7	11.3
7·Glu$_{6d}$[a]	3.5	∼0	4.8	7.8	6.0	−	−
8[b]	3.6	∼0	2.8	6.8	6.4	5.5	8.8
9[b]	3.8	∼0	2.0	∼0	6	6	11.5
10[b]	4.1	∼0	2.4	∼0	2.4	2.4	m.
11[c]	3.6	∼0	2.8	2.6	∼0	5.1	8.8
12[c]	4.0	∼0	2.4	9.5	6.0	3.5	9.0
α-D-Glucopyranose[d]	3.8	9.9	9.6	9.6	2.2	5.5	12.3

[a] In D_2O at pD = 7.4. [b] In DMSO-d_6. [c] In D_2O at pD = 11−12. [d] In D_2O.[52]

coupling constants are contained in Table 3. The $^1J_{C-C}$ coupling constants of the complex **7·Glu** are enclosed in Table 1. The ^{1}H assignments are in agreement with the ^{1}H−^{1}H COSY spectrum and ^{13}C assignments follow from the $^1J_{C-C}$ coupling information. For comparison we have in the Tables included NMR data for selected model compounds (see Scheme 2).

As seen from Table 1 the glucose part of **7·Glu** shows generally high ^{13}C chemical shifts, indicating a furanose form of the glucose.[22] Furthermore, the found $^3J_{H-H}$ values of **7·Glu** exclude a trans diaxial arrangement of H2−H3 and H3−H4 as should be expected for a glucopyranose form. Indeed the measured values correspond to those of structurally related 1,2-bound furanoses (Table 3), $^3J_{H2-H3}$ of ∼0 being especially noteworthy.

For determining the binding sites of the boronic acids, we have recently shown that $^1J_{C-C}$ coupling constants can be used as a very precise indicator.[22,35] The one bond C−C coupling constant, within a RO−C−C−OR′ fragment containing sp^3-hybridized carbon atoms, depends (i) on the O−C−C−O dihedral angle and (ii) on the R−O−C−C torsion's the latter showing the greater variations. According to calculations by Serianni et al.,[36] a minimum value of $^1J_{C-C}$ should be expected for an approximately all eclipsed geometry within such a fragment. In agreement with this prediction we have shown that exceptionally low $^1J_{C-C}$ values are found when the two neighboring carbons are contained in five-membered 1,3-dioxolane- or 1,3,2-dioxaborolane rings.[22,35] For such five-membered rings $^1J_{C-C}$ is found between 33 and 39 Hz whereas for vicinal diols, in general, values lie between 40 and 55 Hz.

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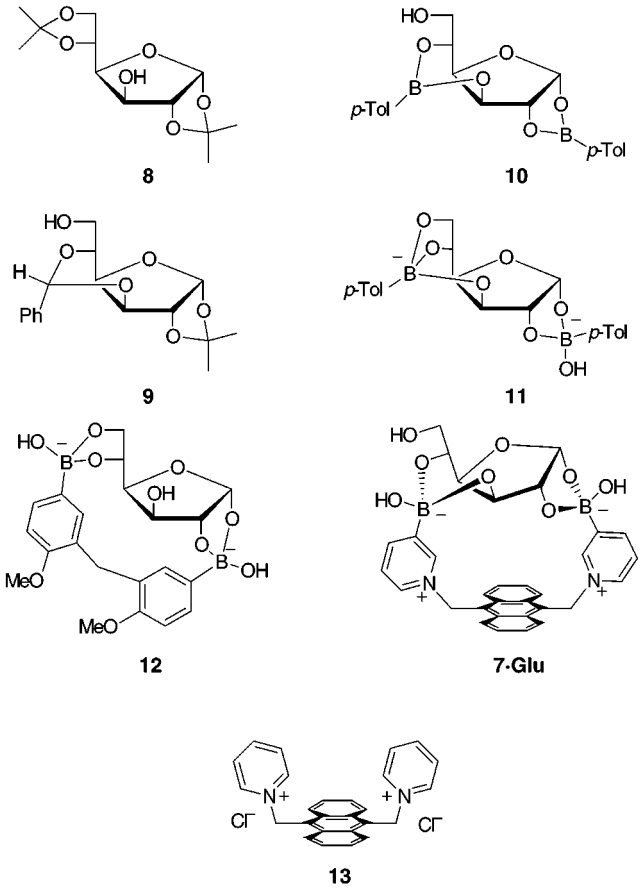
Scheme 2



For a 1:1 complex between a bisboronic acid and a α-glucofuranose, three bisdentate binding modes are conceivable: (i) 1,2:3,5; (ii) 1,2:5,6, and (iii) 1,2:3,5,6. Due to the trans vicinal relationship of O-2 and O-3, no binding is possible here and a seven-membered 1,3,2-dioxaborepane ring, as between O-3 and O-6, is not to be considered in protic media and moreover only when the formation of five- or six-membered rings are excluded.[6,37,38] Inspection of the $^1J_{C-C}$ coupling constants in Table 1 reveals a low constant of 35.9 Hz for $^1J_{C1-C2}$ of **7·Glu** as compared to the values for the free glucopyranoses. Similar low constants are measured in model compounds **8**−**11**. This is consistent with C-1 and C-2 being contained within a five-membered ring, thus giving the one binding site of complex **7·Glu** as 1,2. The high value of $^1J_{C5-C6}$ of 43.5 Hz in **7·Glu** (compare to $^1J_{C5-C6}$ of **8** and **11**) excludes C-5 and C-6 to be part of such a five-membered ring, thus leaving the 3,5 binding site as the only possible for the second boronic acid moiety. Further evidence for the 3,5 binding site was obtained from an experiment with 6-deoxy-L-glucose[39] which formed a 1:1 complex named **7·Glu$_{6d}$** that showed ^{13}C and ^{1}H NMR data almost identical to those of **7·Glu** when chemical shifts were corrected for the 6-deoxy functionality (see Tables 1−3).

On the basis of the above facts, we assign the structure of **7·Glu** to be a 1,2:3,5 bound α-D-glucofuranose. In

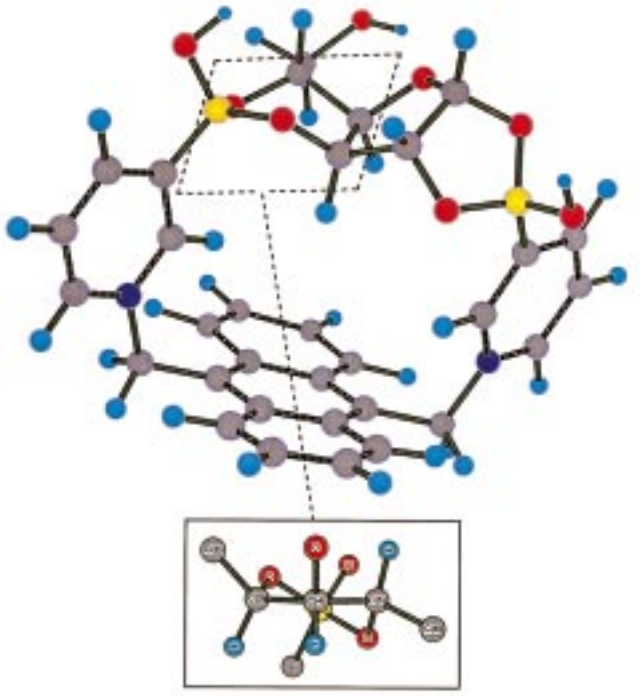


Figure 3. AM-1-calculated structure of complex **7·Glu** and the conformation of the borinane ring around the 3,5 binding site. The calculation was performed with the CS MOPAC/ChemOffice 4.5 program from CambridgeSoft.

Figure 3 an AM-1-calculated structure of **7·Glu** is presented. Inspecting the ^{1}H NMR data of **7·Glu** (and **7·Glu$_{6d}$**), we observed very low chemical shift values of especially H-3 and H-4 but also of H-6a/b (Table 2). This increased shielding can be justified by the proposed structure, as H-3 and H-4 are pointing directly into the anthracene π-cloud with a distance to the anthracene plane of ∼4 Å. In addition, H-4 is even closer to one of the pyridine rings (3.4 Å). Similarly H-6a/b lie within the same average distance (∼4 Å) to the anthracene plane.

Comparing the measured $^3J_{H-H}$ coupling constants of **7·Glu** and **7·Glu$_{6d}$** with those of the structurally related model compounds **9** and **10**, one observes a large deviation of $^3J_{H4-H5}$ (Table 3). However, the structure in Figure 3 shows an approximate skew conformation of the 3,5-borinane ring (see Figure 3) as compared to the well-established chair conformation for the 1,3-dioxane ring in **9** and related sugar derivatives.[40] From the structure in Figure 3, the H5−C5−C4−H4 dihedral angle is found to 155° which from the Karplus relation ($J = 9.5\cdot\cos^2\Phi - 0.28$)[41] corresponds to a $^3J_{H4-H5}$ coupling constant of 7.5 Hz in agreement with the measured high value of 7.3 Hz.

The binding constant of **7·Glu** has been roughly estimated from the ^{1}H NMR intensities of the complex signals compared to those of free glucose and free complex. The values thus obtained give log $K = 4$. From the more precise fluorescence and potentiometric measurements below, slightly lower values were obtained (see below).

Fluorescence Measurements. The above proof of the formation of the well-defined 1:1 complex (**7·Glu**) between bisboronic acid **7** and glucose at neutral pH led naturally to an investigation of the fluorescence response of **7** upon addition of glucose. The absorption spectrum of **7** in a phosphate buffer at pH = 7.4 was measured

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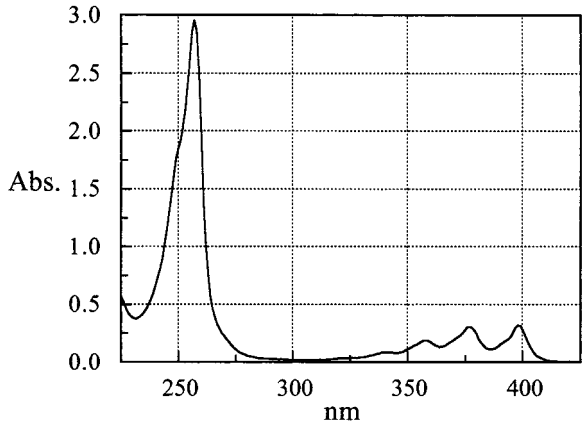

Figure 4. Absorption spectrum of **7** (10^{-5} M) in 0.05 M aqueous phosphate buffer pH 7.4.

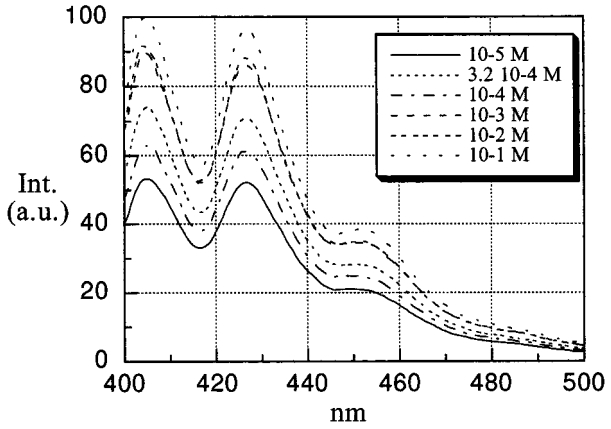

Figure 5. Emission spectrum of **7** (10^{-5} M, 0.05 M aqueous phosphate buffer, pH 7.4) for varying glucose concentration (λ_{ex} = 377 nm).

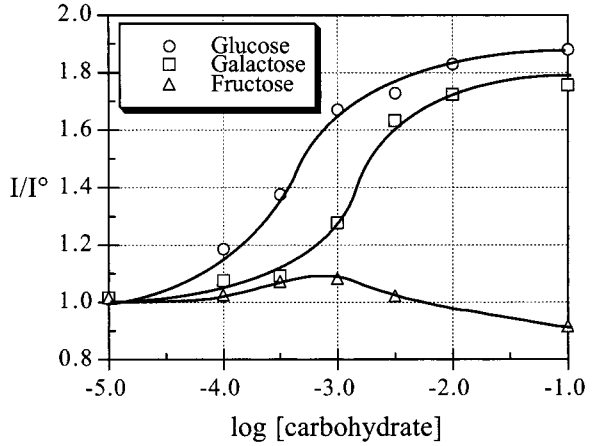

Figure 6. Relative fluorescence of **7** (10^{-5} M, 0.05 M aqueous phosphate buffer, pH 7.4) as a function of carbohydrate concentration. ○ = glucose, □ = galactose, Δ = fructose, λ_{ex} = 377 nm, λ_{em} = 427 nm.

and is shown in Figure 4. The emission spectra (λ_{ex} 377 nm) for varying glucose concentrations are included in Figure 5. Figure 6 shows the relative fluorescence at 427 nm as a function of the carbohydrate concentration. The figure includes the responses of the three most abundant carbohydrates in human blood, namely glucose (~5 mM), galactose (~0.05 mM), and fructose (~0.05 mM). Upon

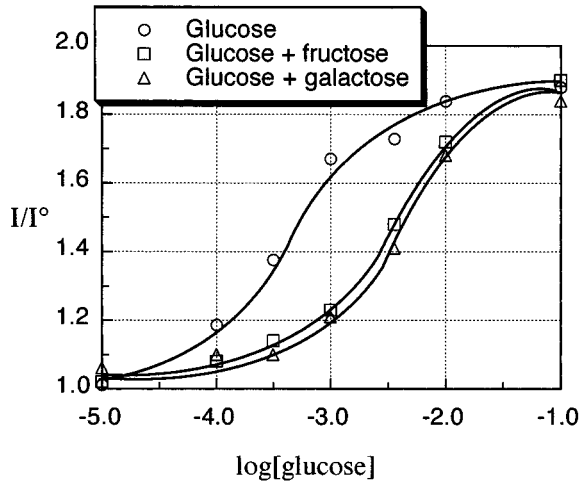

Figure 7. Fluorescence titration of bisboronic acid **7** (10^{-5} M, 0.05 M aqueous phosphate buffer, pH 7.4) with glucose in the presence of either fructose (10^{-4} M) or galactose (10^{-4} M).

addition of glucose an increased fluorescence is observed in accordance with the formation of **7·Glu** being more fluorescent than the noncomplexed bisboronic acid **7**. The relative fluorescence increase upon binding is somewhat less than for a comparable bisboronic acid sensor based on photoinduced electron transfer (PET) quenching as previously reported by Shinkai's group.[28,29]

The measured fluorescence levels are unchanged for 12 h after which we observe a slight decreasing tendency. By plotting $I°/(I - I°)$ against [glucose]$^{-1}$ a stability constant for the 1:1 complex[42] was found to be log K = 3.4. The fluorescence responses of **7** to galactose and fructose, respectively, did not confirm the presence of single 1:1 complexes in accordance with preliminary NMR studies, which in the galactose case show at least two complexes and for fructose display an even more complex situation. The structure of the galactose complexes is currently being investigated in more detail.

To investigate the selectivity of the sensor, competing experiments were performed. Bisboronic acid **7** was titrated with glucose in the presence of fructose and galactose, respectively. The results, which are shown in Figure 7, show less sensitivity to glucose in the presence of 0.1 mM of either carbohydrate in agreement with the observed (nonspecific) complex formation with these sugars. The found selectivity is less than reported for Shinkai's PET sensor;[28,29] however, the concentration ranges (see above) of glucose compared to both fructose and galactose in human blood should exclude the interference from the latter species in the case of blood glucose sensing.

The relative large fluorescence of **7** might be surprising in the light of earlier investigations by several workers[43–45] who have shown the pyridinium moiety in closely related systems (without the boronic acid group) to act as an electron sink for the singlet anthracene. Thus we pre-

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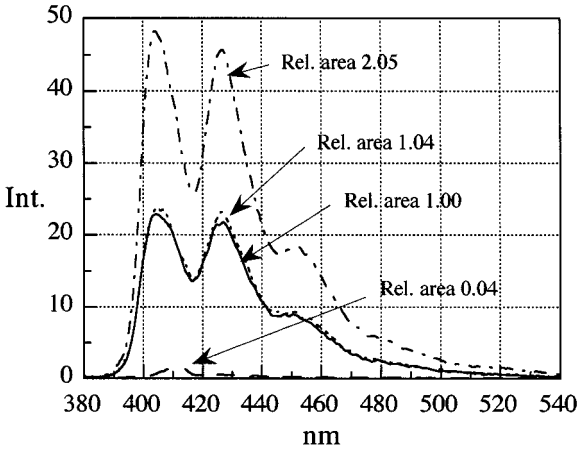
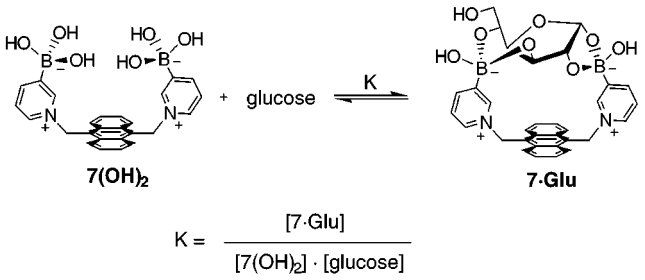


Figure 8. Emission spectra of **7**, **7·Glu**, and **13** in 0.05 M aqueous phosphate buffer, pH 7.4 (no exlusion of oxygen). – · – ·: **7** (0.01 mM) + glucose (0.1 M); - - - -: **7** (0.01 mM) + glucose (0.01 mM); —: **7** (0.01 mM); – – –: **13** (0.01 mM).

pared 9,10-bis(1-pyridiniomethyl)anthracene dichloride **13**[46,47] (Scheme 2) as the "nonboronic acid" analogue of **7**. Figure 8 shows the emission spectrum ($\lambda_{ex} = 360$ nm) of **13** compared to the spectra of bisboronic acid **7** (0 mM and 0.01 mM glucose) and the **7·Glu** complex (0.1 M glucose). As clearly seen from the figure, the pyridinium rings of **13** completely quench the fluorescence, the relative quantum yield being less than 4% of free **7**. This agrees with the behavior of a monopyridinium analogue.[45] It follows that the boronic acid groups of **7** strongly alter the electronic structure of the pyridinium rings, suppressing their acceptor ability. This may be anticipated in view of the exceptionally low pK_a value of the present system (see below) compared to aryl analogues which underlines the large stabilization of the zwitterionic structure.

Binding of glucose enhances the quantum yield with a factor of ~2 relative to nonbound **7**. Several reasons for this observed increase in fluorescence, such as increased rigidity and altered conformation, may be suggested, but a more profound investigation goes beyond the scope of this work. The fact that very little fluorescence change is observed upon addition of fructose may be due to the known favorable 1:1 boronic acid to fructofuranose complexation[35] which suppresses the formation of a similar conformationally restricted bisdentate complex with the sensor molecule.

Potentiometric Titrations. The pK_a values of bisboronic acid **7** was determined by potentiometric titration. In accordance with the reported low pK_a value of *N*-methyl-3-pyridiniumboronic acid (pK_a = 4.4)[34] we found p$K_{a1} = 3.7 \pm 0.1$ and p$K_{a2} = 4.7 \pm 0.1$. These values are in accordance with the requirements for complex formation at neutral pH as discussed in the Introduction. We further determined the formation constant for the complex **7·Glu** as described in the Experimental Section. We found log $K = 3.4 \pm 0.1$ referring to the equilibrium as depicted in Figure 9. This value is in full agreement with the value obtained from the fluorescence measurements, and the binding constant is comparable to a related anthracene bisboronic acid disclosed by Shinkai and co-workers.[28]



$$K = \frac{[7\cdot Glu]}{[7(OH)_2] \cdot [glucose]}$$

Figure 9. Equilibrium between **7** and glucose to give **7·Glu**. The equilibrium constant *K* refers to the measured value.

Conclusions

We have synthesized a new bisboronic acid **7** which combines low pK_a values and water solubility with a structurally optimized design for selective glucose binding. Our binding studies in aqueous solution evidence a strong binding of glucose as the α-D-glucofuranose–1,2:3,5-bisboronate complex at physiological pH. A selective fluorescence response to glucose compared to fructose and galactose is observed which may suggest a similar design for a future sensor of glucose in blood or subcutaneous tissue.

We believe that further optimization of the structural design may lead to new water soluble bisboronic acid sensors which, unlike the **7·**Glu complex, will include binding of all free hydroxy groups of the glucofuranose molecule, i.e., formation of α-D-glucofuranose–1,2:3,5,6-bisboronate complexes. We have recently shown this type of α-D-glucofuranose bisboronates to be the preferred binding mode for glucose both in cases of complexation with a monoboronic acid[22] and with a bisboronic acid (synthesized by Shinkai's group).[30] In our opinion binding of the more abundant α-pyranose form of glucose by boronic acids is not to be considered in the future design of boronic acid based sensors for aqueous systems.

Experimental Section

General. ^{1}H and ^{13}C NMR spectra were recorded at 25 °C at 300 and 75 MHz, respectively. Chemical shifts are reported in ppm. The spectra are referenced as follows: D_2O, ^{13}C, and ^{1}H referenced internally to DSS; CD_3OD, ^{13}C referenced internally to CD_3OD = 49.0 ppm and ^{1}H to CHD_2OD = 3.30 ppm; acetone-d_6, ^{13}C referenced internally to CD_3COCD_3 = 29.8 ppm and ^{1}H referenced internally to CD_2HCOCD_3 = 2.05 ppm. Evaporations were performed in vacuo on a rotary evaporator at 40–50 °C. Melting points are uncorrected. Microanalyses were performed by Service Central d'Analyses, CNRS, Vernaison, France.

Materials. All chemicals used were of reagent grade, and all solvents were of HPLC grade.

NMR Experiments for Structure Elucidation. Equimolar amounts of **6** and carbohydrate were dissolved in D_2O. The pH was adjusted to 7.4 with 0.1 M NaOD and the sample diluted to give a final concentration of **6** and carbohydrate of 35 mM each. ^{1}H and ^{13}C NMR spectra were recorded at 25 °C at 400 and 100 MHz, respectively. The $^1J_{C-C}$ coupling constants of the complex **7·Glu** was obtained on a sample prepared from **6** and uniformly $^{13}C_6$ labeled α-D-glucose. The $^1J_{C-C}$ coupling constants of model compounds **8** and **9** were measured in natural abundance using the INADEQUATE technique. The remaining $^1J_{C-C}$ coupling constants of Table 1 were obtained from ^{13}C NMR spectra of samples prepared with uniformly ^{13}C labeled glucose.

Fluorometry. Fluorescence spectra are recorded 20 min after mixing. Carbohydrate solutions are allowed to equilibrate before use. No precautions to exclude oxygen were taken.

Potentiometric Methods. Measurements of pH were made under argon in an thermostated reaction chamber and with a combination glass electrode. Standardizations were performed using standard buffer solutions at pH 4.01, 6.86, and 10.01 (certified buffers). Potentiometric determinations of pK_a values were accomplished in 10^{-3} M solutions of ligand (and glucose in case of binding constant determination) which were 0.1 M in NaCl. Freshly titrated NaOH solution (0.01 M) were used in all titrations. The pK_a values were obtained from dublicate titrations, and the binding constants were determined by the pH-shift method of Torssell[48] and Lorand and Edwards[7] using the Superquad II program.[49]

9,10-Bis(chloromethyl)anthracene (1). Prepared according to Miller et al.[50] *Caution:* This compound is highly allergenic and should be handled with care. ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.40 (4H; anthracene-H1,4,5,8), 7.60 (4H; anthracene-H2,3,6,7), 5.70 (4H, s, CH_2). Mp 254−256 °C (dec). MS: M^+: 274 *m*/*z*. Anal. Calcd for $C_{16}H_{12}Cl_2$: C, 69.84; H, 4.40. Found: C, 69.66; H, 4.26.

3-Pyridineboronic acid (2).[33,51] Dry THF (200 mL) was placed under Ar in a dry three-necked, 500 mL bottle equipped with magnetic stirrer, thermometer, and addition funnel. The solvent was cooled to −40 °C, and BuLi (2.7 M in heptane, 42 mL, 0.11 mol) was added. The mixture was further cooled to −100 °C, and 3-bromopyridine (16.0 g, 0.10 mol) in dry THF (35 mL) was added over 0.5 h, keeping the temperature at −100 °C. The resulting green solution was allowed to heat to −80 °C for 15 min and then recooled to −105 °C. Trimethyl borate (17 mL, 0.15 mol) was added as quickly as possible keeping the temperature below −80 °C. A green-brown precipitate forms, and the suspension was stirred for a further 2 h at −80 °C and was then allowed to slowly reach room temperature. The resulting orange solution was stirred overnight and then recooled to 0 °C and hydrolyzed with water (30 mL). The pH was adjusted to pH ≈ 6 with HCl (4 M), and THF was evaporated in vacuo. The orange aqueous solution was continuously extracted with ether for two weeks and the ether extract evaporated. The crude **2** (10.4 g), probably containing some $B(OH)_3$, was recrystallized from boiling MeOH to yield 3.9 g (32%) of the free acid containing a little dimethyl ester as seen from the IR spectrum. Another 2.8 g (23%) was obtained from evaporation of the mother liquor and recrystallization. This material was used in the next step without further purification.

5,5-Dimethyl-2-(pyridine-3-yl)-1,3,2-dioxaborinane (3). 3-Pyridineboronic acid (see above) (**2**, 1.00 g, ∼8.1 mmol) and 2,2-dimethyl-1,3-propandiol (0.87 g, 8.4 mmol) were dissolved in dioxane (70 mL). To remove water, dioxane (40 mL) was slowly distilled off at atmospheric pressure, and the remaining solvent was evaporated in vacuo. The resulting white powder was dissolved in boiling dioxane (12 mL), and hot cyclohexane (40 mL) was quickly added. Slow cooling yielded **3** as a white microcrystalline material which was isolated and washed with a small amount of cyclohexane. Drying in a vacuum yielded 1.34 g (87%). A small sample was sublimated (15 mmHg, 90 °C) for analysis. mp 94−95 °C. ^{1}H NMR (acetone-d_6) δ 8.84 (dd, 1H, $J_{2,4} \approx 1$ Hz, $J_{2,5} \approx 1$ Hz, H-2), 8.59 (dd, 1H, $J_{5,6} = 4.9$ Hz, $J_{4,6} = 2.0$ Hz, H-6), 8.01 (ddd, 1H, $J_{4,5} = 7.6$ Hz, H-4), 7.30 (ddd, 1H, H-5), 3.83 (s, 4H, CH_2), 1.03 (s, 6H, CH_3). ^{13}C NMR (acetone-d_6, *C*−B not observed due to quatropolar relaxation) δ 155.5, 152.3, 141.8, 123.8, 72.8, 32.5, 21.8. Anal. Calcd for $C_{10}H_{14}BNO_2$: C, 62.87; H, 7.39; N, 7.33. Found: C, 62.55; H, 7.48; N, 7.31.

9,10-Bis[*N*-[3-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)pyridinium]methyl]anthracene Dichloride (4). The protected pyridineboronic acid (**3**, 400 mg, 2.09 mmol) and 9,10-bis(chloromethyl)anthracene (**1**, 287 mg, 1.04 mmol) was suspended in dry CH_3CN (15 mL) under Ar and refluxed for 15 h. The solvent was removed in vacuo, and the resulting light-yellow powder was dissolved in MeOH (10 mL). The solution was filtered from some insoluble impurities and reevaporated to a yellow glass (0.68 g, ∼100%) which was deprotected as described in the next step without further purification.

1,3-[Anthracene-9,10-diyl[bis[methyl[*N*-(pyridinium-3-yl)]]]]-1,3-dimethoxydiboroxane Dichloride (6). Crude **4** (0.68 g, 2.09 mmol) was deprotected by stirring in acetone−water (9:1, 10 mL) for 4 h. Some lumps were manually cracked. The suspension was transferred to a centrifuge glass with additional acetone (5 mL) and centrifuged. The light-yellow precipitate was washed with acetone (4×) and dried in vacuo to yield crude **5** (543 mg). Compound **5** was evaporated with MeOH (10 mL, 3×). The yellow-orange crystalline compound thus obtained was recrystallized by dissolving in boiling MeOH (13 mL) followed by carefully addition of ether (15 mL). Slow cooling, filtration, and washing with ether−MeOH yielded **6** as slightly hygroscopic orange needles. Drying in vacuo over P_2O_5 gave (**6**, 395 mg, 72%). Mp > 230 °C (dec). ^{1}H NMR (CD_3OD) δ 9.12 (s, 2H, H−d), 8.74 (d, 2H, $J_{e/g,f} = 7.6$ Hz, H−e or H−g), 8.70 (d, 2H, $J_{e/g,f} = 6.3$ Hz, H−e or H−g), 8.51 (m, 4H, H−a), 7.96 (dd, 2H, H−f), 7.77 (m, 4H, H−b), 7.08 (s, 4H, H−c), 3.34 (s, 9H, 3 x CH_3OH). ^{1}H NMR (D_2O) δ 8.67 (br, 6H), 8.27 (br, 4H), 7.91 (br, 2H), 7.76 (br, 4H), 3,40 (s, 9H). ^{13}C NMR (CD_3OD) δ 151.8, 149.1, 145.0, 137 (br, C−B), 133.1, 129.9, 128.9, 127.3, 125.4, 57.6, 49.8 (masked). Anal. Calcd for $C_{29}H_{31}B_2Cl_2N_2O_{4.5}$ (**6**·MeOH·$^1/_2H_2O$): C, 60.88; H, 5.46; N, 4.90, Cl, 12.39. Found: C, 60.82; H, 5.63; N, 4.88, Cl, 12.46.

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