The Structure of the Sugar Residue in Glycated Human Serum Albumin and Its Molecular Recognition by Phenylboronate

Jan Rohovec,[a, b] Thomas Maschmeyer,[a] Silvio Aime,[c] and Joop A. Peters*[a]

Abstract: Quantification of the extent of glycation of human serum albumin (HSA) and of haemoglobin provides a record of average mid- and long-term blood-sugar concentrations, respectively; this is very useful for the management of diabetes. The reaction of D-glucose with propylamine affords the corresponding Schiff base, *N*-propylamino-D-glucoside, in the cyclic form. This compound is not stable: upon standing or treatment with acid it is converted, by an Amadori rearrangement, into *N*-propylfructosamine. Both amino sugars occur predominantly in the β-pyranose form. Phenylboronate forms highly stable boronate esters through binding of the *cis* 1,2-diol moiety in the furanose form of *N*-propylfructosamine. Between pH 5 and 10, an electrostatic interaction between the protonated amino group and the negatively charged boronate moiety affords an additional stabilisation of the ester. The Schiff base, however, has no observable interaction with phenylboronate. In aqueous solution the Schiff base is in equilibrium with propylamine and glucose. Upon addition of phenylboronate, this equilibrium shifts to the side of glucose due to the formation of highly stable phenylboronate esters of the β-furanose form of this compound. After Amadori rearrangement, the sugar moieties in glycated human serum albumin have a similar structure, they occur as an equilibrium of the β-pyranose (59%), α-furanose (19%) and β-furanose (24%) anomers. The open form was not observed. The β-furanose anomer is selectively recognised by phenylboronate.

Keywords: diabetes · glycoproteins · molecular recognition · NMR spectroscopy · phenylboronic acid

Introduction

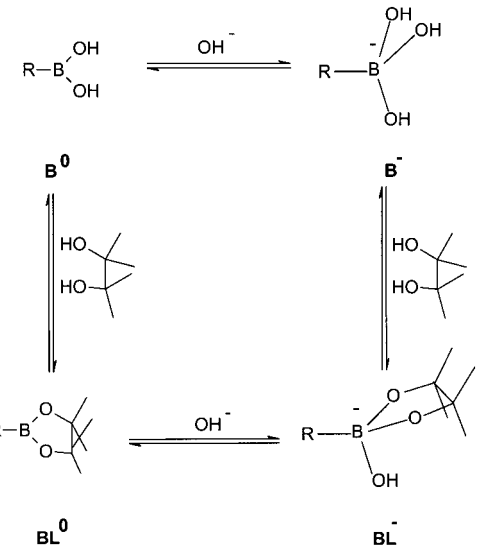
Boric acid and boronic acid are known to form stable esters with polyols and saccharides. These compounds have gained increased attention because of the possibility of exploiting them for the molecular recognition of sugars.[1–3] Boronic acids have been used in the construction of glucose-sensitive receptors and give a response, for example, as a signal in the visible spectrum[4, 5] or as a change in the luminescence behaviour of the receptor.[6–11] Furthermore, an assay for the degree of glycation of serum has been developed; it consists of phenyl boronate conjugated to a Gd^{III} chelate and responds to the recognition of the sugar residue by increasing the ^1H NMR relaxation rate of the bulk water.[12]

[a] Dr. J. A. Peters, Dr. J. Rohovec, Prof. Dr. T. Maschmeyer
Laboratory for Applied Organic Chemistry and Catalysis
Delft University of Technology, Julialaan 136
2628 BL Delft (The Netherlands)
Fax (+31) 15-27-84289
E-mail: j.a.peters@tnw.tudelft.nl

[b] Dr. J. Rohovec
Department of Inorganic Chemistry, Universita Karlova
Albertov 2030, 128 40 Prague 2 (Czech Republic)

[c] Prof. Dr. S. Aime
Dipartimento di Chimica I.F.M., Università di Torino
via Pietro Giuria 7, 10125 Torino (Italy)

The advantages of boronates ($\mathbf{B}^-$, see Scheme 1) for the recognition of polyols are that the bonds which it forms with the polyol substrates (**L**) are covalent and that the formation and the hydrolysis of boronate esters ($\mathbf{BL}^-$, also called boronate complexes) are rapid in aqueous solution. The



Scheme 1. Equilibria between boronic acids and diols.

 DOI: 10.1002/chem.200204632

equilibrium concerned is strongly pH dependent since polyols react preferentially with boronate anions ($\mathbf{B}^-$). Consequently, the equilibrium shifts to the side of the boronate esters ($\mathbf{BL}^-$) upon increase of the pH.[13, 14]

A favourable interaction between the polyol substrate and the boronate anions requires a vicinal diol moiety that is able to reach a *cis* arrangement. This recognition reaction results in a relatively stable five-membered 1,3,2-dioxaborolane ring.

Analysis of the structures of the borate and boronate esters of sugars may be complicated by the formation of esters with a boronate/sugar stoichiometry higher than one and by the occurrence of the sugars in various anomeric forms. Previous research has demonstrated that local stability constants of the borate esters of sugars have the following stability sequence: *cis*-1,2-diol furanose $\gg$ exocyclic 1,2-diol pyranose $>$ exocyclic 1,2-diol furanose $>$ *cis*-1,2-diol pyranose $>$ exocyclic *cis*/*trans*-4,6-diol pyranose $\gg$ *trans*-1,2-diol pyranose/furanose.[15] Studies on phenylboronate esters of glucose suggest that they behave similarly.[10, 13]

Up to now, the main efforts towards the recognition of saccharides have been focussed on glucose.[1, 2, 4, 5–11, 16] The interactions of glucose with phenylboronate derivatives appear to be similar to those with borate. Glucose reacts with phenylboronate anions in basic media to form esters with boron/saccharide stoichiometries of 1:1 and 2:1.[15, 17–19] The thermodynamically most stable species all have five-membered bor(on)ate ester rings.

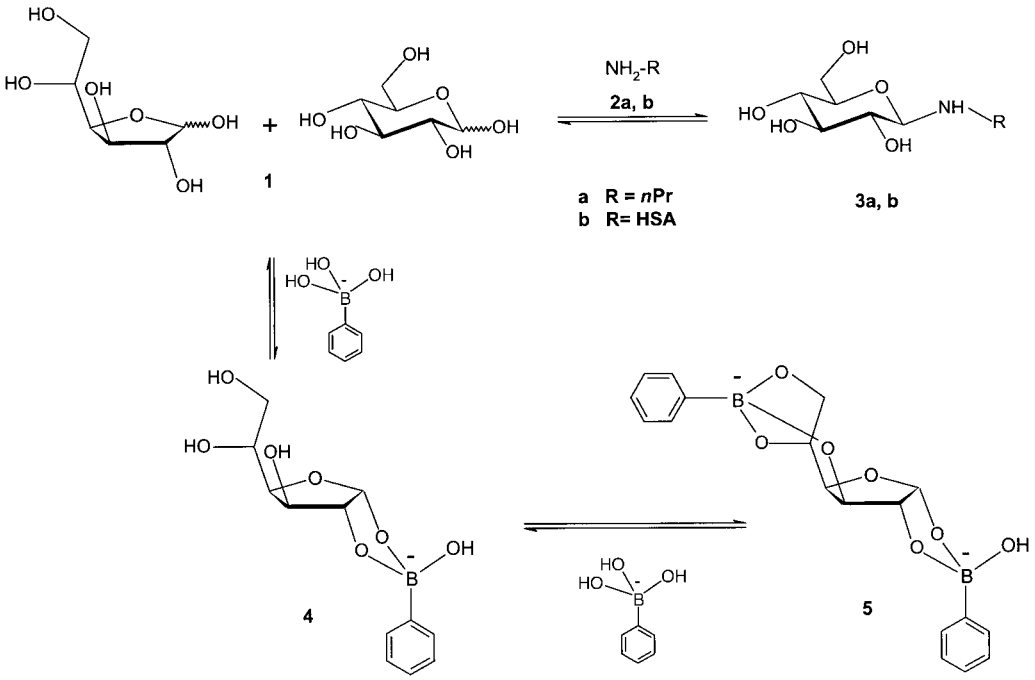
Only a few studies on the interaction of phenylboronic acids with aminosaccharides have been reported.[20, 21] Aminosaccharides are of biological and medical importance as changes in the structure and concentration of sugar residues in glycoproteins are often related to diseases. For instance, the high glucose levels in the blood of patients with diabetes mellitus leads to increased nonenzymatic glycation of serum and haemoglobin-protein free amino groups. This glycation is associated with pathological complications, including cardiovascular diseases and blindness.[22, 23] In the glycation of a peptide, initially glucose reacts with a free amino group to afford a Schiff base, which then undergoes an irreversible Amadori rearrangement to a fructosamine product.[24, 25] Quantification of the extent of glycation of human serum albumin (HSA) and of haemoglobin provides a record of average mid- and long-term blood-sugar concentrations,[24] respectively; this is very useful for the management of diabetes.[26] Molecular recognition of fructosamine is a key issue and, therefore, the design of optimal diagnostic tools requires insight into the precise structure of this moiety in glycated proteins.

Until now, neither the structure of the sugar residue of glycated HSA nor that of simple N-alkylfructosamines has been elucidated. Generally, these residues are represented in their acyclic forms.

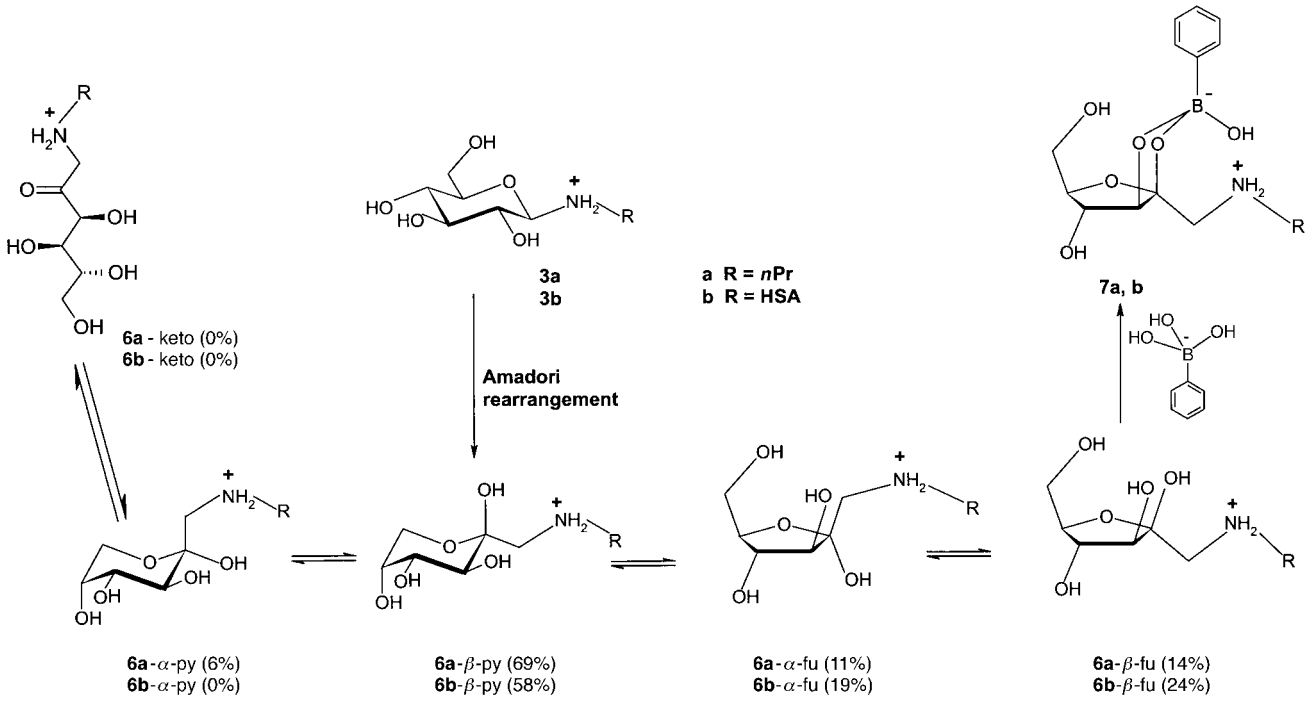
Here, we report the results of a study on the glycation and the subsequent Amadori rearrangement of HSA by using uniformly ^{13}C-labelled D-glucose. The structures of the sugar residue in the intermediate and in the rearranged product are elucidated with ^{13}C NMR spectroscopy. Furthermore, the interactions with phenylboronate were investigated. To facilitate the interpretation of the data, similar experiments were performed with *N*-propylaminoglucoside and *N*-propylfructosamine as simple low-molecular-weight models. The stabilities of the phenylboronate esters of the latter compound were studied in detail.

Results and Discussion

The Schiff base of D-glucose and propylamine, *N*-propylamino-D-glucoside (3a): Treatment of glucose (**1**) with propylamine (**2a**, see Scheme 2) affords the corresponding Schiff



Scheme 2. Glycation of HSA and propylamine.



Scheme 3. Amadori rearrangement of the Schiff bases **3a** and **3b**, followed by reaction with phenylboronic acid. The molar ratios of the products formed are given in parentheses, accuracy: from **3a** ±1%, from **3b** ±5%.

base, *N*-propylamino-D-glucoside (**3a**).[27, 28] No solvent was applied in this reaction. Schiff base **3a** is stable at −20 °C for at least a year. However, ^{13}C NMR spectra reveal that, in aqueous solution, compound **3a** is present as an equilibrium mixture with D-glucose and propylamine. A slow irreversible Amadori rearrangement of **3a** into *N*-propylfructosamine (**6a**) was observed in a 0.25 M solution of the Schiff base. After one week at pH 7.4 and room temperature, the sample contained 32 mol% Schiff base **3a**, 29 mol% D-glucose (**1**), 29 mol% propylamine (**2a**) and 10 mol% of the rearrangement product **6a** (see Scheme 3). The ^{13}C NMR spectra showed that Schiff base **3a** occurred almost exclusively in a single anomeric form. Its ^{13}C resonances were assigned by means of a ^{13}C–^{13}C COSY spectrum of a sample of **3a** with a uniformly ^{13}C-labelled glucose part. A comparison of the chemical shifts (see Table 1) with those of known saccharides[29] indicates a β-pyranose form of **3a**. This is confirmed by the $^3J_{HH}$ coupling constants for H1–H2 and H2–H3, which are 8.9 and 8.7 Hz, respectively. These values are typical for *trans* diaxial arrangements of protons in pyranose forms; for furanose forms coupling constants <4 Hz should be expected.

The interaction of **3a** with phenylboronate was studied at pH 9–11 by ^{13}C NMR spectroscopy by using freshly prepared samples. The signals observed were assigned by comparison with spectra of D-glucose (**1**), *N*-propylfructosamine (**6a**) and the phenylboronate complexes of D-glucose[18] at the same pH. Only the 1:1 and 1:2 esters of α-D-glucofuranose and phenylboronate (**4** and **5**, see Scheme 2) were observed, the equilibrium between Schiff base **3a** and glucose (**1**)/propylamine (**2a**) is shifted completely to the side of glucose by the complexation of the latter by phenylboronate. The hydrolytic

Table 1. Chemical shifts of compounds in solutions of **6a**, its phenylboronate esters and **6b** in D_2O (0.125 M) at 25 °C.

	6a-β-py		**6a**-α-fu		**6a**-β-fu				**6b**[a]
	HL	L	HL	L	HL	L	BHL	BL	L
C(1)	54.3	56.5	53.7	–[b]	53.7	–[b]	53.3	54.6	54.7
C(2)	96.6	100.4	100.3	108.1	103.4	108.1	109.9	112.7	96.6
C(3)	71.2	71.7	83.8	–[b]	84.1	–[b]	84.7	83.9	70.8
C(4)	70.5	–[b]	75.6	–[b]	79.3	–[b]	80.6	81.1	70.8
C(5)	70.8	70.5	77.5	–[b]	82.6	–[b]	86.4	85.6	70.8
C(6)	65.4	68.2	62.3	–[b]	63.4	–[b]	66.1	66.4	69.2
C(7)[c]	51.6	52.7	51.4	–[b]	51.4	–[b]	52.0	52.9	–[d]
C(8)[c]	20.3	23.3	20.1	–[b]	20.1	–[b]	21.4	23.9	–[d]
C(9)[c]	11.6	12.5	11.6	–[b]	11.6	–[b]	11.9	12.6	–[d]
B	–	–	–	–	–	–	−11.8	−12.0	–
N[e]	−344.8	−348.5	−344.0	–[b]	−344.5	–[b]	−347.7	–[b]	–[b]

[a] The ^{13}C chemical shifts given were measured on a compound with ^{13}C-labeled sugar moieties. [b] Observation was difficult due to line-broadening and low intensity. [c] C atoms of the *N*-propyl group. [d] Not observed due to low intensity, these nuclei were not ^{13}C labelled. [e] Assignments of the α- and β-furanose forms may be interchanged.

reaction of **3a** in the presence of phenylboronate was faster than in its absence, but no attempt was made to quantify this. The high preference of phenylboronate for glucose can be ascribed to the high degree of preorganisation for phenylboronate ester formation of the 1,2-*cis*-diol moiety in its α-furanose form. Such a *cis*-diol is absent in the corresponding anomer of Schiff base **3a**, due to the presence of the *N*-propylamino group at the C1 position in that species.

***N*-propylfructosamine (6a)**: This compound was prepared as the oxalate salt by the Amadori rearrangement of **3a** in dioxane according to the procedure described by Michael and Hagemann.[28] The ^{13}C NMR spectrum of aqueous solutions of **6a** shows four sets of resonances (see Table 1). The assignment of the signals in the anomeric region (90–110 ppm) was carried out by comparison with the known chemical shifts of anomeric carbons of fructose derivatives.[29] All other saccharide resonances were assigned with the use of a $^{13}C-^{13}C$ COSY experiment on a sample of **6a** in which the fructose moiety was uniformly labelled with ^{13}C. Integration of the resonances for the anomeric ^{13}C nuclei in a quantitative ^{13}C experiment shows the anomeric equilibrium to consist of 6 mol% α-pyranose, 69 mol% β-pyranose, 11 mol% α-furanose and 14 mol% β-pyranose (see Scheme 3). This molar ratio remains constant between pH 1 and 8.5. At higher pH values, measurement was hampered by excessive line broadening. A similar behaviour has been observed with fructose and can be ascribed to base catalysis of the mutarotation. No C=O resonance was detected over the pH range 1–13; this indicates that the amount of open form is negligible. The assignment of the β-pyranose form as being the major anomer is supported by the ^{1}H NMR spectrum, which showed a large coupling between H-3 and H-4 (9.4 Hz); this is in agreement with the axial–axial arrangement of the protons concerned in the pyranose form. The dihedral angles in the furanose forms would give rise to relatively small vicinal proton–proton coupling constants (<4 Hz).

The interaction between the oxalic acid salt of *N*-propylfructosamine **6a** and phenylboronic acid in aqueous solution was studied at 25 °C as a function of the pH by using 0.25 M solutions with molar ratios **6a**/$PhBO_2H_2$ of 1:1, 1:2 and 1:4. Between pH 2.9 and 5, the ^{11}B spectra displayed a signal at −11.5 ppm besides the signal for the equilibrium between phenylboronic acid and phenylboronate (see above). The ^{13}C chemical shifts in the region 0–120 ppm were identical to those of the spectrum of **6a** in the absence of phenylboronic acid; this indicates that, in this pH region, no interaction occurs between phenylboronic acid and **6a**. The signal at −11.5 ppm can therefore be ascribed to a phenylboronate ester of oxalic acid; this was confirmed by separate ^{11}B NMR measurements on a sample of oxalic acid and phenylboronic acid. It should be noted that the ^{11}B chemical shift indicates that the B atom in this compound is tetragonal. For the trigonal complex, which has been studied in organic solution, a ^{11}B chemical shift of 17 ppm has been reported.[30]

This study also revealed that the complex concerned readily hydrolyses upon increase of the pH; at pH >5, it was no longer observed. A reaction between **6a** and phenylboronic acid begins at pH 5, as demonstrated by the appearance of a new signal at −11.9 ppm in the ^{11}B NMR spectrum and one in the anomeric part of the ^{13}C NMR spectrum at 109.9 ppm. Upon further increase of the pH, the new resonances increase in intensity at the expense of those of free phenylboronic acid/phenylboronate and free **6a**, respectively. Apparently, the exchange between free **6a** and its phenylboronate esters is slow on the ^{13}C NMR timescale in this case. The intensities of the resonances for the anomeric carbon atoms in quantitative ^{13}C NMR spectra indicate that the stoichiometry of the esters is 1:1. Only one set of new signals was observed upon formation of the boronate ester; this leads to the conclusion that a single phenylboronate ester species is present. The chemical shift of the anomeric carbon atom is known to be a sensitive probe for distinguishing between the pyranose and furanose forms of sugars. The ^{13}C chemical shift of the anomeric carbon of the phenylboronate ester ($\delta = 109.9$ ppm at pH 5.9) points to a furanose form. This should be the β-form, since only that furanose form has a *cis*-diol function; phenylboronate ester formation on *trans*-diol functions is very unfavourable for steric reasons. The other ^{13}C resonances of this phenylboronate ester were assigned with the use of a $^{13}C-^{13}C$ COSY experiment on **6a**, uniformly labelled with ^{13}C in the saccharide part. The chemical shifts of the other C atoms are similar to those of the corresponding nuclei of the furanose forms and higher than those of the pyranose forms of free **6a** (see Table 1), fructose and glucose. The difference between the ^{13}C chemical shifts of **6a** and its phenylboronate ester, **7a**, is about the same as that between β-D-glucofuranose and its phenylboronate ester.

Further support for the furanose configuration of the phenylboronate ester **7a** was obtained from the vicinal H–H couplings in the ^{1}H NMR spectrum, which were all smaller than 3 Hz.

The observed chemical shifts of the ^{11}B nucleus (−11.9 ppm) and of the ^{15}N nucleus (−347.7 ppm) are in the typical regions for a tetrahedral boron surrounded by O atoms and for a ^{15}N atom in a protonated amine. No evidence for formation of a B–N bond was found. The ^{11}B chemical shift mentioned above indicates that the amino N atom is not involved in a dative bond, since in that case an ^{11}B NMR chemical shift between −4 and −7 ppm would be expected.[31–33] The absence of a dative bond between the N and the B atoms is also in agreement with previous studies on the borate esters of various amino diols[34] and can be rationalised by the steric strain that would be introduced upon formation of that bond. The optimal length for a N → B bond is typically 1.6–1.7 Å,[35] whereas this separation is at least 4 Å in the most favourable conformation of the boronate ester of **6a**.

It should be noted that in contrast to the phenomena observed with the system phenylboronic acid/3-(*N*,*N*-dimethylamino)-1,2-propandiol (**8**),[36] only a single set of ^{13}C NMR signals is observed for the phenylboronate ester **7a**; this suggests that one of the two possible diastereomers is predominant. Most likely, this can be ascribed to the electrostatic interaction between the negative boronate group and the positively charged ammonium group, which is minimised in the enantiomer **7a** with an *S* configuration around the B atom, in which the B–OH group is pointing in the direction of the ammonium group.

The formation of species with a saccharide/phenylboronate stoichiometry of 1:2 is very unfavourable for the β-furanose form. Only in the presence of a large excess of phenylboronate at pH > 13.5, is a bisboronate species formed. A broad resonance was observed for the anomeric carbon at 102 ppm with a intensity of about 10 % of that of the anomeric carbon of the 1:1 phenylboronate ester **7a**. Considering the sterically dictated preference of phenyl boronate esters for *cis*-diol functions, the phenylboronate functions should be bound at the 2,3- and the 4,5-*cis*-diol functions of the β-D-pyranose anomer.

Glycated human serum albumin: To investigate the structure of the sugar residue in glycated HSA we used ^{13}C NMR spectroscopy on HSA glycated with D-glucose uniformly labelled with ^{13}C. The glycation was achieved by incubation of HSA (**2b**) and [^{13}C]-glucose in water at 65 °C for 4 hours, followed by dialysis.[37] Unfortunately, the high intensities of the ^{13}C resonances of the labelled free glucose (present in excess) made it impossible to monitor the glycation reaction and the subsequent Amadori rearrangement by ^{13}C NMR spectroscopy. Two samples of HSA with different degrees of glycation (containing 13 and 6 saccharide units per molecule of HSA) were prepared. Both samples gave identical ^{13}C NMR spectra (see Figure 1); this showed that the degree

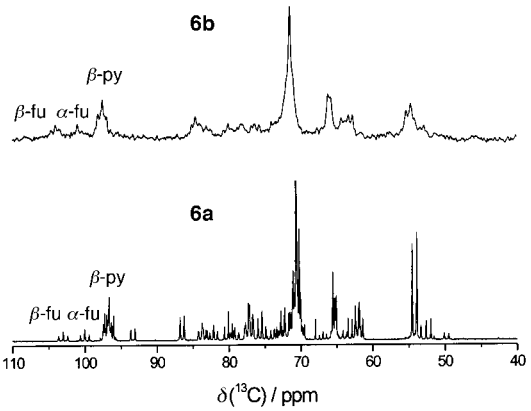
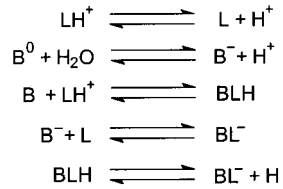


Figure 1. ^{13}C NMR spectra of **6a** and **6b** enriched in the saccharide part by ^{13}C.

of glycation has no influence on the structure of the sugar residue. The region between 85 and 120 ppm was well resolved and showed three resonances at 97.2, 100.7 and 103.7 ppm with a line-width of about 10 Hz. By comparison with the spectra of *N*-propylfructosamine (**6a**, see above), these resonances could be assigned to the anomeric carbon atoms of β-pyranose (**6b**-β-py), α- (**6b**-α-fu) and β-furanose (**6b**-β-fu) forms, respectively (see Scheme 3). Integration of these resonances in the ^{13}C NMR spectrum showed that the molar ratio of these anomers to be about 58:24:19. A resonance for the anomeric carbon of the α-pyranose form could not be detected, probably because it is hidden under the relatively broad signal of the β-pyranose anomeric carbon. No carbonyl signals were observed; this indicates that also here the amount of the acyclic form (analogously to **6a**-keto) is negligible. Once again, addition of phenyboronate resulted exclusively in a phenylboronate ester of the β-furanose of **6b** (**6b**-β-fu).

Stability of the phenylboronate esters: Based on the structures of the species described above, the set of equilibria depicted in Scheme 4 should be considered to describe these systems.



Scheme 4. Equilibria involved in binding of aminopolyols and phenylboronate. $B^0 = PhB(OH)_2$, $B^- = [PhB(OH)_3]^-$, L = **6a**, LH^+ is the *N*-protonated form of L, BL^- and BLH are the corresponding phenylboronate esters.

The associated stability constants are defined by Equations (1) – (5).

$$K_1 = [L][H^+]/[LH^+] \quad (1)$$

$$K_2 = [B^-][H^+]/[B^0] \quad (2)$$

$$K_3 = [BLH]/[LH^+][B^-] \quad (3)$$

$$K_4 = [BL^-]/[B^-][L] \quad (4)$$

$$K_5 = [BL^-][H^+]/[BLH] = K_1K_4/K_3 \quad (5)$$

For compounds **6a** and **b**, L denotes the anomeric form under consideration.

The various stability constants were obtained by fitting experimental curves of ^{13}C and ^{11}B chemical shifts as a function of pH and of the integrals in the ^{11}B NMR resonances with values for these parameters calculated from speciations obtained from Equations (1) – (4) and mass balances. The chemical shifts of the nuclei in the protonation equilibria were calculated as the weighted averages of the chemical shifts in the protonated and unprotonated forms.

The protonation constants K_1 and K_2 were fixed at the values determined by fitting ^{13}C chemical-shift titration curves of the free amines concerned and the ^{11}B chemical-shift titration curve of a sample of phenylboronic acid, respectively. The value of K_2 was determined to be 8.86; this is in agreement with literature values.[38]

The values obtained for the various stability constants are compiled in Table 2, and Figure 2 shows a comparison of the experimental and calculated parameters for the NMR data for the formation of the phenylboronate ester of compound **6a**. For comparison data on the system phenylboronic acid/3-(*N*,*N*-dimethylamino)propan-1,2-diol (**8**) are also included in this table. The large difference between the stability constants of the phenylboronate esters of **8** and the β-furanose anomer of **6a** can be rationalised by the highly preorganised *cis*-diol function in the latter compound. The values of K_3 for **6** and **8** demonstrate the dramatic effect of preorganisation on the

Table 2. Equilibrium constants for the equilibria involved in the phenylboronate ester formation of compounds **3a, 6a** and **8**.

Compound	anomeric form	$\log K_1$	$\log K_3$	$\log K_4$	$\log K_5$[a]
3a	β-pyranose	-6.67 ± 0.03	–	–	–
6a	β-pyranose	-10.40 ± 0.01	–	–	–
	β-furanose	-10.6	5.19 ± 0.02	4.40 ± 0.16	-11.4 ± 0.2
8[b]	–	-9.76 ± 0.09	2.20 ± 0.05	1.33 ± 0.02	-10.6 ± 0.2

[a] $\log K_5 = \log K_1 + \log K_4 - \log K_3$. [b] Included for completeness.[36]

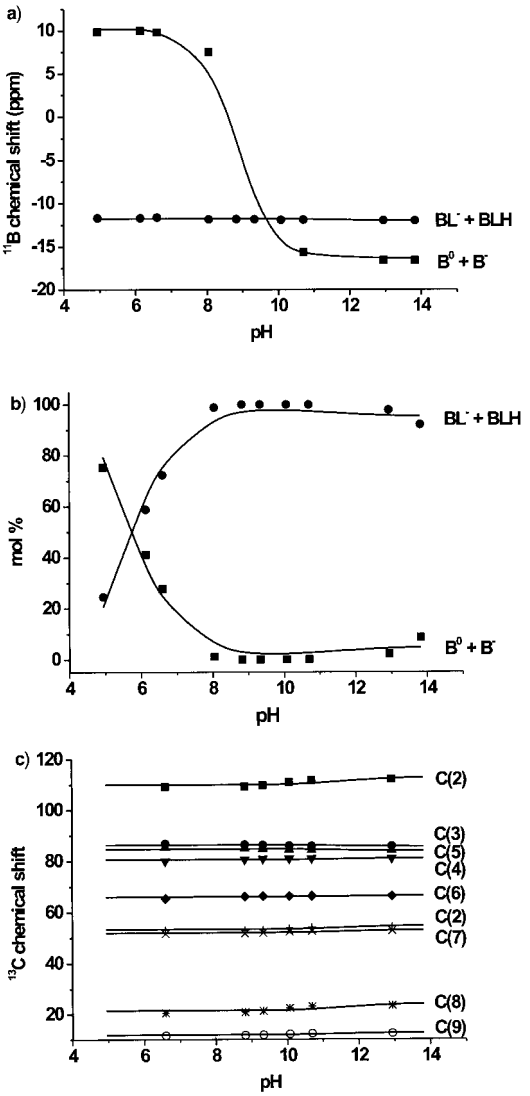


Figure 2. Comparison of the experimental and calculated parameters for the NMR data for the formation of the phenylboronate ester of compound **6a**.

stability of the complexes. A comparison of the values of K_3 and K_4 clearly shows the stabilising of the phenylboronate ester upon protonation of the amine function; for both compounds this amounts to an order of magnitude. An important aspect in relation to molecular recognition of aminosaccharides is the strong interaction between phenylboronate and **6a** even at physiological pH. This can be explained by the coulombic attraction of the negatively charged B unit and positively charged ammonium group of the aminosaccharide under those conditions applied.

The above-mentioned stability constants are consistent with phenylboronate binding of **6a** with a high selectivity over glucose. This was confirmed by a competition experiment in which phenylboronic acid (0.25 M) was added to an equimolar mixture of D-glucose and **6a** (0.25 M each). The ^{13}C NMR spectrum of this mixture (see Figure 3) shows that the ratio of phenylboronate esters of **6a** to those of glucose was larger than 10.

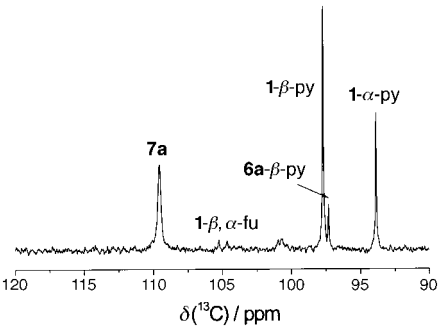


Figure 3. ^{13}C NMR (anomeric region) of the competition experiment: phenylboronic acid (0.25 M), D-glucose (0.25 M) and **6a** (0.25 M), pH 8.2.

Conclusion

The results presented demonstrate that boronates are highly suitable for the molecular recognition of saccharides. Particularly, strong interactions can be expected for sugars that are able to form β-furanose rings. This explains, for example, the successful application of a conjugate of a Gd^{III} chelate and phenylboronic acid for the determination of the extent of glycation of HSA,[12] and the success of the affinity chromatographic system based on *m*-aminophenylboronic acid to separate fructosamines from urine.[39]

Insight into the structure of the sugar residue in glycated HSA may be helpful for the explanation of the hyperglycaemia-induced pathogenesis of vascular complexation of diabetes. Furthermore, we expect that improved understanding of the sugar-residue structure as well as its interaction with the artificial receptor, that is, phenylboronic acid, may be useful for the development of new and improved diabetes diagnostics.

Experimental Section

Materials and methods: Uniformly ^{13}C-labelled D-glucose was obtained from Aldrich–Sigma and used without further purification. All other reagent-grade chemicals were purchased from commercial sources and used without further purification.

***N*-propylamino-D-glucoside (3a)**: This compound was prepared by following the procedure of Michael and Hagemann.[28] A compound with a uniformly labelled saccharide moiety was prepared in a similar way starting from uniformly ^{13}C-labelled D-glucose. The crude compound was recrystallised from 1,4-dioxane. M.p. 90 °C; ^{13}C NMR (75.5 MHz, [D$_6$]DMSO): 91.49, 78.33, 78.15, 74.23 71.25, 62.07, 48.20, 23.81, 12.49.

***N*-aminopropylfructosamine oxalate (6a)**: *N*-aminopropylfructosamine was prepared by using the procedure reported by Michael and Hage-

mann.[28] A compound with a uniformly labelled saccharide moiety was prepared in a similar way starting from compound **3a** with a uniformly ^{13}C-labelled D-glucose unit. M.p 130 °C (dec.); ^{13}C NMR (75.5 MHz, [D_6]DMSO): 165.48, 95.80, 81.91, 75.91, 69.23, 60.69, 50.92, 49.57, 18.55, 11.02.

Glycated HSA (6b): Glycation of HSA (**2b**):[37] D-glucose (**1**) uniformly labelled with ^{13}C (99 %, 50 mg) was dissolved in water (0.9 mL) at 56 °C. To this solution HSA (80 mg) was added in one portion. The reaction was monitored by measuring the absorbance at 530 nm of samples after addition of nitroblue tetrazolium chloride in carbonate buffer.[40] The equilibrium was reached after about 4 h. Then the excess of glucose was removed by dialysis against water for 3 days at 5–8 °C by using a benzoylated cellulose tubing. The dialysed solution was lyophilised to yield glycated HSA as a white solid (**6b**). The extent of glycation as determined by mass spectrometry and colorimetrically with the use of nitroblue tetrazolium chloride[40] was 13 saccharide units per HSA molecule. In a similar way a sample of HSA with six saccharide units was prepared.

Physical methods: ^1H (300 MHz), ^{13}C (75.5 MHz), ^{11}B (96.2 MHz) and ^{15}N (30.4 MHz) NMR spectra were recorded on a Varian INOVA-300 spectrometer with 5 mm sample tubes. Chemical shifts are reported as δ values. For measurements in D_2O, *tert*-butyl alcohol was used as an internal standard with the methyl signal calibrated at 1.2 ppm (^1H) or 31.2 ppm (^{13}C). Quantitative ^{13}C NMR experiments were performed with a 45° flip angle, an acquisition delay of 30 s and gated decoupling. ^{11}B chemical shifts are reported with respect to 0.1M boric acid in D_2O as external standard,[41] and ^{15}N chemical shifts are reported with respect to neat nitromethane as external standard. In both cases, the substitution method was applied to measure these shifts. The ^{11}B NMR spectra were processed by using linear prediction to remove baseline distortions due to glass resonances. Peak positions and intensities were determined by fitting the observed signal with a Lorenzian line function. ^{15}N NMR spectra of acidic samples were recorded by using DEPT techniques. Because of line broadening, this technique was not effective for basic samples; therefore, these samples were measured by using a one-pulse sequence with gated decoupling.

The NMR samples were prepared in D_2O/H_2O (10:90). The pH of samples was measured at ambient temperature by using a Corning 125 pH meter with a calibrated micro-combination probe purchased from Aldrich. The pH values reported are direct meter readings (no correction for D-isotope effects was made). pH values were adjusted by using dilute solutions of NaOH and HCl.

Acknowledgement

This work was performed in the framework of the EU COST Action D18 "Lanthanide Chemistry for Diagnosis and Therapy".

[1] T. D. James, K. R. A. S. Sandanayake, S. Shinkai, *Angew. Chem.* **1996**, *108*, 2038; *Angew. Chem. Int. Ed. Engl.* **1996**, *54*, 2093.
[2] G. Wulff, *Pure Appl. Chem.* **1982**, *54*, 2093.
[3] T. D. James, S. Shinkai, *Top. Curr. Chem.* **2002**, *218*, 160.
[4] K. R. A. S. Sandanayake, S. Shinkai, *J. Chem. Soc. Chem. Commun.* **1994**, 1083.
[5] C. J. Ward, P. Patel, P. R. Ashton, T. D. James, *Chem. Commun.* **2000**, 229.
[6] T. D. James, K. R. A. S. Sandanayake, R. Iguchi, S. Shinkai, *J. Am. Chem. Soc.* **1995**, *117*, 8982.
[7] T. D. James, K. R. A. S. Sandanayake, R. Iguchi, S. Shinkai, *Angew. Chem.* **1994**, *94*, 2287; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 2207.
[8] T. D. James, P. Linnane, S. Shinkai, *J. Chem. Soc. Chem. Commun.* **1996**, 281.
[9] D. P. Adhikiri, M. D. Heagy, *Tetrahedron Lett.* **1999**, *40*, 7893.
[10] H. Eggert, J. Frederiksen, C. Morin, J. C. Norrild, *J. Org. Chem.* **1999**, *64*, 3864.
[11] W. Yang, H. He, D. G. Drueckhammer, *Angew. Chem.* **2001**, *113*, 1764; *Angew. Chem. Int. Ed.* **2001**, *40*, 1714.
[12] S. Aime, M. Botta, W. Dastrú, M. Fasano, M. Panero, *Inorg. Chem.* **1993**, *32*, 2068.
[13] J. P. Lorand, J. O. Edwards, *J. Org. Chem.* **1959**, *24*, 769–774.
[14] M. van Duin, J. A. Peters, A. P. G. Kieboom, H. van Bekkum, *Tetrahedron* **1984**, *40*, 2901.
[15] R. van den Berg, J. A. Peters, H. van Bekkum, *Carbohydr. Res.* **1994**, *253*, 1.
[16] J. C. Norrild, I. Søtofte, *J. Chem. Soc. Perkin Trans. 2* **2002**, 303.
[17] G. de Wit, PhD Thesis, Delft University of Technology, The Netherlands, **1979**.
[18] J. C. Norrild, H. Eggert, *J. Am. Chem. Soc.* **1995**, *117*, 1479.
[19] M. Bielecki, H. Eggert, J. C. Norrild, *J. Chem. Soc. Perkin 2* **1999**, 449.
[20] C. R. Cooper, T. D. James, *Chem. Lett.* **1998**, 883.
[21] C. R. Cooper, T. D. James, *J. Chem. Soc. Perkin 1* **2000**, 963.
[22] R. J. Koenig, S. H. Blobstein, A. Cerami, *J.Biol. Chem*. **1977**, *252*, 992.
[23] M. A. M. van Boekel, *Mol. Biol. Rep*. **1991**, *15*, 57.
[24] D. A. Armbruster, *Clin. Chem. (Washington, DC, U.S.)* **1987**, *33*, 2153.
[25] J. F. Day, S. R. Thorpe, J. W. Baynes, *J. Biol. Chem.* **1979**, *254*, 595.
[26] The Diabetes Control and Complications Trial Research Group, *N. Engl. J. Med.* **1993**, *329*, 977.
[27] F. Michael, A. Frowein, *Chem. Ber.* **1957**, *90*, 1599.
[28] F. Michael, G. Hagemann, *Chem. Ber.* **1959**, *92*, 2836.
[29] K. Bock, C. Peresen, *Adv. Carbohydr. Chem. Biochem.* **1983**, *41*, 27.
[30] H. Nöth, B. Wrackmeyer, *Nuclear Magnetic Resonance of Boron Compounds*, Springer, Berlin, **1978**.
[31] T. Burgemeister, R. Grobe-Einsler, R. Grotstollen, A. Mannschreck, G. Wulff, *Chem. Ber.* **1981**, *114*, 3403.
[32] M. Biedrzycki, W. H. Scouten, Z. Biedrzycka, *J. Organomet. Chem.* **1992**, *431*, 255.
[33] J. C. Norrild, *J. Chem. Soc. Perkin Trans. 2*, **2001**, 719.
[34] J. van Haveren, J. A. Peters, J. G. Batelaan, A. P. G. Kieboom, H. van Bekkum, *Recl. Trav. Chim. Pays-Bas* **1989**, *108*, 179.
[35] H. Höpfl, *J. Organomet. Chem.* **1999**, *581*, 129.
[36] L. Frullano, J. Rohovec, K. Djanashvili, T. Maschmeyer, J. A. Peters, unpublished results.
[37] K. Ikeda, Y. Sakomoto, Y. Kawasaki, T. Miayake, K. Tanaka, T. Urata, Y. Katayama, S. Ueda, S. Horiuchi, *Clin. Chem.* **1998**, *44*, 256.
[38] G. E. K. Branch, D. L. Yabroff, B. Bettmann: *J. Am. Chem. Soc.***1934**, *56*, 937.
[39] M. Brownlee, H. Vlassara, A. Cerami, *Diabetes* **1980**, *29*, 1044.
[40] R. N. Johnson, P. A. Metcalf, J. R. Baker, *Clin. Chim. Acta* **1983**, *127*, 87.
[41] Conversion to $BF_3 \cdot Et_2O$ scale is as follows: $\delta(H_3BO_3$ scale$) = \delta(BF_3 \cdot Et_2O$ scale$) + 18.7$.

Received: December 3, 2002 [F4632]

