An illusive chiral aminoalkylferroceneboronic acid. Structural assignment of a strong 1 : 1 sorbitol complex and new insight into boronate–polyol interactions

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The structure of a strong complex between D-sorbitol and (*S*,*S*)-2-(*N*,*N*-dimethyl-1-aminoethyl)ferroceneboronic acid (**1**) has been determined by NMR spectroscopy. On the basis of ^{1}H, ^{13}C and ^{11}B NMR spectral data, including information from COSY, NOESY and HSQC two-dimensional experiments, a 2,3,5-bound sorbitol complex is deduced. On the basis of coalescence phenomena observed when varying the temperature and pH, an equilibrium between a complex with a covalent and a coordinative B–O5 bond, respectively, is elucidated, and a possible stabilisation of the latter by hydrogen bonding to the amino group is discussed. The existence of intramolecular B–N bonds in **1** and its complexes has been evaluated but no evidence of such bonds was obtained.

Introduction

The interaction between sorbitol and boric acid has been known for many years as a means of increasing the acid strength of boric acid to allow boron determination by titration methods. A few attempts have been made to determine the structure of the obviously *very* strong complex(es) formed,[1,2] but the formation of mixtures of bischelated spiro-complexes has made the investigations difficult and unsuccessful.[2] To our best knowledge no attempts have been made to elucidate the structure of sorbitol complexes with boronic acids in aqueous solution. The boronic acids obviously cannot make the entangling spiro-complexes.

Lately we have been interested in the properties of the chiral ferroceneboronic acid (**1**, Fig. 1) which has been suggested by Shinkai's group as an electrochemical carbohydrate sensor.[3] This compound was obviously designed to be capable of effective binding of diols at neutral pH due to the expected formation of an intramolecular B–N bond. This kind of interaction had proven useful in several benzeneboronic acid-based sensors,[4–8] as, even at neutral pH, it can give a (partial) tetrahedral boron atom, which is a prerequisite for strong binding.

The results from Shinkai's study were, however, not very promising in that they only obtained a very weak response to D-glucose at pH = 7. Nevertheless a modest binding of **1** to D-fructose and an even stronger binding to D-sorbitol was reported.

We had several reasons to conduct further studies on this system, especially as the B–N interaction in the ferroceneboronic acid (**1**) seemed, for reasons given below, highly questionable. Firstly, we had for some time been curious about the large difference in the binding constants of fructose and sorbitol. Among carbohydrates fructose is known to form one of the strongest boronic acid complexes, and our previous studies on the structure of this complex had shown the formation of a preferred tridentate β-D-fructofuranose 2,3,6-tri-*O*-(arylorthoboronate) at high pH.[9] A recent study has indicated that this complex is the sole species present at neutral pH-values,[10] so this further suggests that sorbitol should be able to form an even superior tridentate complex. These speculations, however, surely exclude a B–N interaction within these complexes.

Secondly, a simple consideration of the different bond lengths and bond angles in the ferroceneboronic acid (**1**) leads to the conclusion that the B–N distance in the non-perturbed system would be approximately 2.5 Å, whereas the optimal distance is 1.6–1.7 Å,[11] making a B–N bond highly unlikely. Even though considerable distortions of the bonding angles in several benzene derivatives with an intramolecular B–N bond have been established in the solid phase,[12] no evidence of a direct B–N interaction was obtained when we recently performed a crystallographic study of **1** and some close analogues.[13]

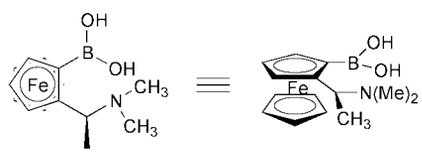

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Fig. 1 Structure of (*S*,*S*)-2-(*N*,*N*-dimethyl-1-aminoethyl)ferroceneboronic acid (**1**).

Thirdly, it was found that the binding constants of the oxidised form of **1** were more than one order of magnitude larger than those of the non-oxidised form. This could correlate with a strong dependence on the pK_a value of the isolated boronic acid group, which should not be as pronounced in cases where a strong B–N interaction exists.

Finally, we were curious to learn about the structure of the expected 1 : 1 sorbitol–boronic acid complex, from which we might obtain a better insight into the structural factors that affect and enhance the stability of boronic acid–diol complexes.

For these reasons we initiated the present study, based on NMR spectroscopy, to clarify the structure of the strong complex between the ferroceneboronic acid **1** and D-sorbitol.

Results

The optically pure (*S*,*S*)-2-(*N*,*N*-dimethyl-1-aminoethyl)ferroceneboronic acid (**1**) was first prepared by Silva and coworkers.[14] However, these authors erroneously designated the compound they prepared as (*S*,*R*), which is not in accordance with the generally accepted nomenclature deduced by Ugi.[15] We have repeated their synthesis, starting from the optically pure (−)(*S*) amine, to verify the stereochemistry and the sign of the reported rotation of the compound. Due to the moderate yield

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Table 1 ^{1}H NMR chemical shifts (ppm) and J_{HH} coupling constants (Hz) of the **1·Sorb** complex[a]

	H-1a 3.61	H-1b 3.70	H-2 4.13	H-3 4.03	H-4 3.45	H-5 3.72	H-6a 3.36	H-6b 3.87	
	$^2J_{1a,1b}$ −10.1	$^3J_{1a,2}$ 8.9	$^3J_{1b,2}$ 5.5	$^3J_{2,3}$ ~0	$^3J_{3,4}$ 4.0	$^3J_{4,5}$ 9.3	$^3J_{5,6a}$ 8.6	$^3J_{5,6b}$ 2.3	$^2J_{6a,6b}$ −11.2
	C*H*–CH$_3$ 4.63 (q, 7 Hz)	Fc^{9}H 4.32[b]	FcH 4.22	FcH 4.21	Fc$_{unsub}$H 4.16	N–C^{15}H$_3$ 2.83[b]	N–C^{16}H$_3$ 2.27[b]	CH–C*H*$_3$ 1.57 (d, 7 Hz)	

[a] In D_2O–CD_3OD (2 : 1 w/w), pH = 8.56. [b] Assigned from NOEs.

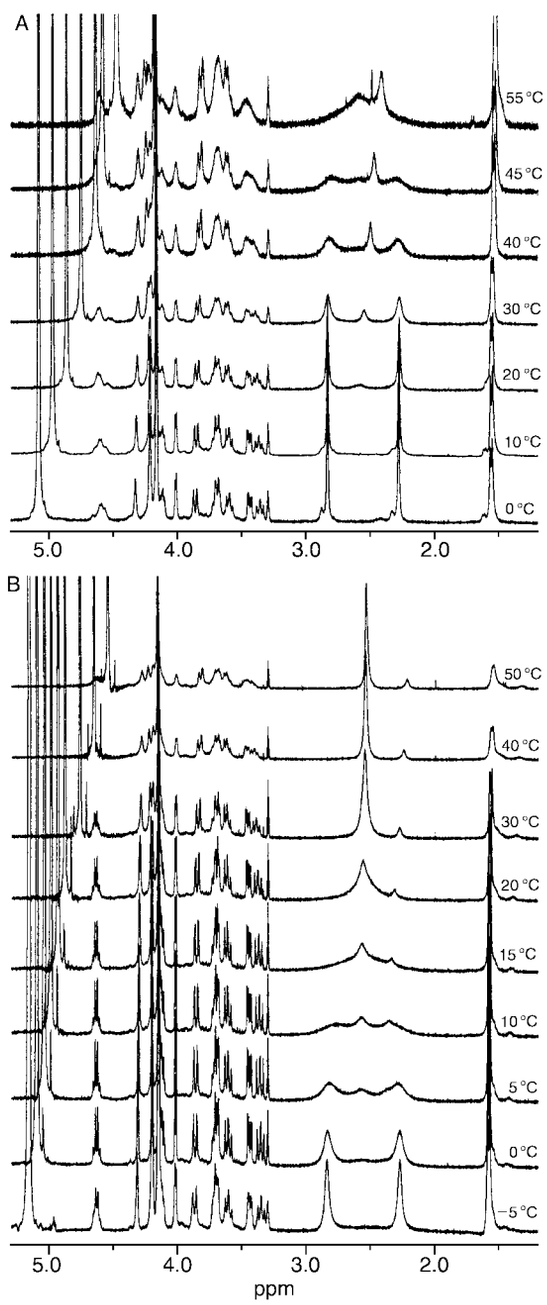


Fig. 2 Temperature dependence of the ^{1}H NMR spectrum of the **1·Sorb** complex in CD_3OD–D_2O (1 : 2 w/w); A: pH = 8.6 and B: pH = 12.3.

obtained by the published method (47%) we slightly modified the procedure. By lithiation with *sec*-butyllithium instead of *n*-butyllithium we were able to obtain (+)(*S*,*S*)-**1** in 72% yield after crystallisation from EtOAc–pentane. As the ferroceneboronic acid (**1**) was not adequately soluble in pure D_2O for the NMR measurements, the complex between D-sorbitol and **1** was studied in a slightly buffered (~65 mM in phosphate) mixture of CD_3OD–D_2O (1 : 2 w/w). A standard 1 : 1 mixture of the components (48 mM each) was prepared and the pH adjusted when needed with a dilute NaOD solution.

The ^{1}H NMR spectrum of the described 1 : 1 mixture was first recorded at pH = 8.6 and 25 °C and latterly over a range of temperatures (See Fig. 2A). The spectrum at room temperature revealed the presence of signals from a single complex (**1·Sorb**); no additional signals from either free sorbitol or the free boronic acid (**1**) were observable (compare with spectrum at 20 °C in Fig. 2A). Compared to the very complicated ^{1}H NMR spectrum of free sorbitol,[16,17] reasonably separated absorptions from the complex at 400 MHz were obtained. From the spectrum two clearly different signals from the two *N*-methyl groups were observable at 2.27 and 2.83 ppm; also a much weaker broad absorption was detected between the two signals. This observation prompted us to run a series of experiments at temperatures ranging from 0 °C to 55 °C. The spectra are depicted in Fig. 2A. At 0 °C a minor set of signals at 1.63, 2.32, 2.88 and 4.91 ppm (~9%) from the protons in the side chain of the ferrocene appeared in addition to those of the major complex (~91%); however no additional lines originating from the alditol part or the ferrocene skeleton were detectable. By heating the sample a complicated coalescence phenomenon was observed which resulted in two separate broad absorptions from *N*-methyl groups at 2.4 and 2.6 ppm in a ratio of ~1 : 2. No major concomitant displacements of the signals from the alditol part were detected but substantial broadening of isolated signals was observed (Fig. 2A).

The ^{1}H and ^{13}C spectral data of the **1·Sorb** complex were then recorded at 0 °C. The data are compiled in Tables 1 and 2. The assignments were obtained from COSY, HSQC and NOESY experiments and refer to the atom numbering given in Fig. 6 (see Discussion section). The geminal and vicinal proton coupling constants could be obtained directly from the 1D spectrum, whereas the $^1J_{CH}$ coupling constants were obtained from the proton coupled HSQC spectrum. The variations of the $^1J_{CH}$ coupling constants will not be discussed in this work. The final assignment of C-1/H-1 *vs*. C-6/H-6 was obtained from an experiment with (1*R*/*S*)-1-deuterio-(UL-$^{13}C_6$)-D-sorbitol made by reduction of the uniformly $^{13}C_6$-labelled glucose with $NaBD_4$ in CD_3OD. The $^1J_{CC}$ coupling constants of the complex were determined for a sample made from uniformly ^{13}C-labelled sorbitol. The $^1J_{CC}$ values of D-sorbitol in D_2O were obtained from a 1D INADEQUATE experiment on non-labelled D-sorbitol.

The proton spectra of the 1 : 1 mixture were recorded at pH = 7.4 and later at pH = 12.3. At pH = 7.4 additional lines originating from sorbitol and from the free ferroceneboronic acid (**1**) appeared. From these data the binding constant was estimated (see later). Studying the spectra at pH = 12.3 at varying temperature a similar situation to the one observed at pH = 8.6 was evident, but the two large singlets from the

Table 2 ^{13}C NMR chemical shifts (ppm) and $^{1}J_{CH}$ coupling constants in parenthesis (Hz) of the **1·Sorb** complex[a]

C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8
64.3[b]	73.4	75.0	67.8	75.2	65.0	70.9	84.8
(142.9; 141.2)[c]	(145.6)	(150.5)	(145.6)	(140.4)	(142.9; 141.1)[c]	(No proton)	(No proton)
C-9	C-10	C-11	C-12	C-13	C-14	C-15	C-16
68.9	69.2[d]	75.0[d]	68.9	63.0	9.52	41.9	34.2
(176.1)	(175.1)	(174.1)	(176.8)	(142.3)	(133.4)	(143.0)	(141.1)

[a] In D_2O–CD_3OD (2 : 1 w/w), pH = 8.56. [b] Assigned from C-1 deuterium labelled D-sorbitol. [c] $^{1}J_{CH}$ to Hb and Ha respectively. [d] C-10 and C-11 may be interchanged.

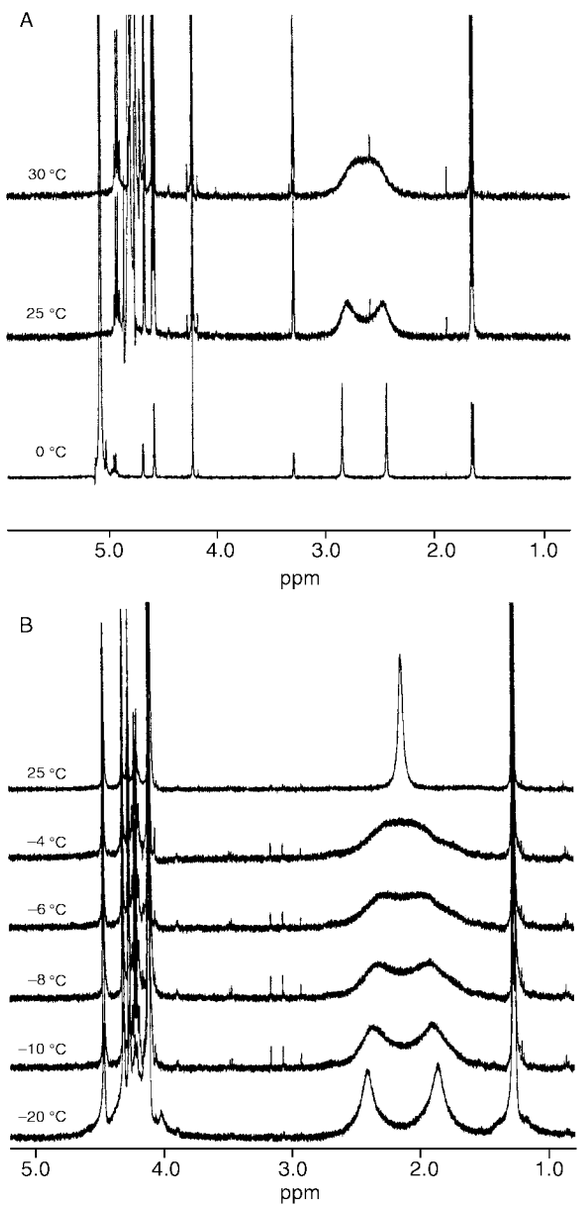


Fig. 3 Temperature dependence of the ^{1}H NMR spectrum of the ferroceneboronic acid (**1**) in A: CD_3OD–D_2O (1 : 2 w/w), pH = 7.4 and B: $CDCl_3$.

N-methyl groups now coalesced to one reasonably sharp absorption upon heating (Fig. 2B).

The coalescence of the free ferroceneboronic acid (**1**) was studied for comparison in both the aqueous buffer (pH = 7.4) and in $CDCl_3$. The spectra are depicted in Fig. 3A and 3B respectively.

^{11}B NMR data were collected at different pH-values for both the **1·Sorb** complex and for the free ferroceneboronic acid (**1**). The data are shown in Fig. 4. We also performed experiments

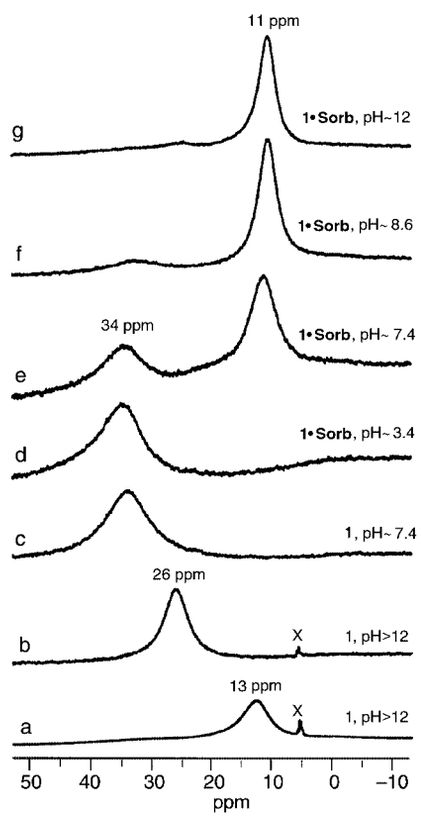


Fig. 4 ^{11}B NMR of **1** and **1·Sorb** in CD_3OD–D_2O (1 : 2 w/w) at varying pH. At high pH partial decomposition to borate (marked ×) is observed.

with added diols and with unsubstituted ferroceneboronic acid for comparison (Fig. 5).

Discussion

Structural assignment

From the data in Tables 1–4 we have assigned the **1·Sorb** complex as the 2,3,5-bound sorbitol as illustrated in Fig. 6. In the following paragraphs we will discuss the evidence for this assignment.

We have previously investigated the complexes formed between boronic acids and D-glucose[7,18,19] as well as D-fructose[9] by NMR spectroscopic methods. These studies have led to the important conclusion that measuring the one-bond carbon–carbon coupling constants can be a powerful tool for structural assignments of these complexes. Whenever the carbon atoms within a vicinal diol fragment were included in a five-membered ring we found, in agreement with calculations from Serianni's laboratory,[20] exceptionally low values of the $^{1}J_{CC}$ coupling constants. For various polyols $^{1}J_{CC}$ coupling constants are typically found between 38–45 Hz, but when the two carbon atoms are within a five-membered ring typical values from 33–37 Hz result.

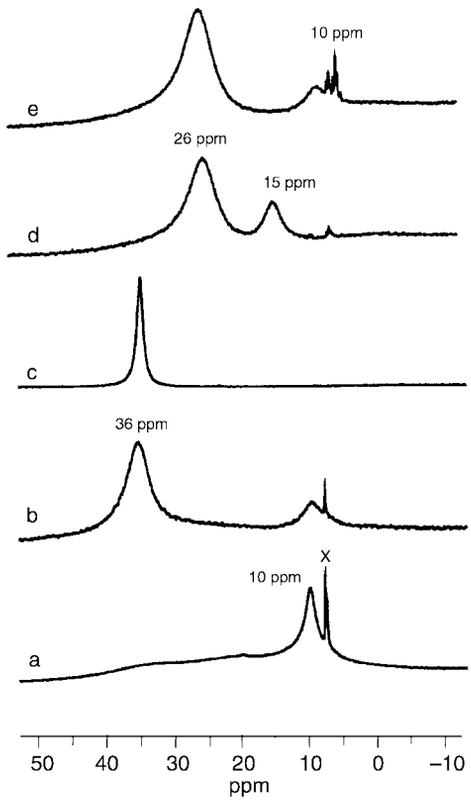


Fig. 5 ^{11}B NMR in CD_3OD of a: ferroceneboronic acid in CD_3OD + dilute aq. NaOD (pH > 12), b: ferroceneboronic acid in CD_3OD + dilute aq. NaOD (pH = 11), c: ferroceneboronic acid in 100% CD_3OD, d: **1** + 5 equiv. pinacol, e: **1** + 5 equiv. 2,2-dimethylpropane-1,3-diol. At high pH partial decomposition to borate (marked ×) is observed.

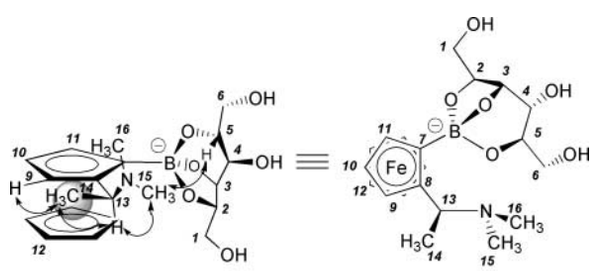


Fig. 6 Assignment and atom numbering of the **1·Sorb** complex. Important NOEs are indicated with double headed arrows.

For the present study the measured values of $^1J_{CC}$ for the **1·Sorb** complex and sorbitol respectively are compared in Table 3. As seen from this table the measured $^1J_{C2\text{-}C3}$ of 36.7 Hz, compared to a value of 41.5 Hz in non-bound sorbitol, clearly establishes that C-2 and C-3 are incorporated in a five-membered ring, so we can unequivocally assign the 2,3 position as the primary binding site. As the 2- and 3-hydroxy groups of D-sorbitol are the only *threo*-oriented diol in sorbitol our assignment complements the results of Van Duin *et al.*, who concluded that internal *threo*-diols make stronger borate complexes than do similar *erythro*-oriented diols.[21]

Shielding effects observed on both ^{1}H and ^{13}C absorptions upon boronate formation may be used for structural determination of the complexes but, from our experience, only with great circumspection. Generally, a deshielding is observed of the ^{13}C atoms within the cyclic borate- and boronate esters,[22–24] but variations with structure are large and in our own studies we find many examples where this general rule fails. However, in Table 4 we have listed a comparison of the ^{13}C chemical shifts of free sorbitol and of the **1·Sorb** complex. As seen from this table, the variations do not give a consistent picture according to the assignment made. The C-2 is shielded by 0.8 ppm whereas C-3 and C-5, on the other hand, are largely deshielded. These data make a conflicting picture around the 2,3-binding site, but may point to 5-OH as the third binding site. The variations of the ^{13}C (de)shielding in the present **1·Sorb** complex may be ascribed to several factors, but particularly the orientation of the polyol with respect to the ferrocene moiety may have a pronounced effect, as one can go from shielding above to deshielding below the cyclopentadienyl rings.[25]

From the above discussion it is obviously not trivial to assign the 5-OH as the third binding site only on the evidence of the large deshielding of C-5 of 3.1 ppm. The ^{1}H chemical shifts do not provide further evidence. Protons H-2 and H-3 are deshielded by +0.34 and +0.24 ppm, respectively, compared to sorbitol in neutral D_2O.[17] Protons H-1a, H-1b and H-5 are within 0.03 ppm, H-4 is −0.18 ppm and H-6a and H-6b are +0.11 and −0.26 ppm, respectively. The deshieldings of H-2 and H-3 are supportive of a 2,3 binding site but H-4/5/6 give an ambiguous picture. Nevertheless, the hydroxy groups of C-1 and C-4 can be excluded as binding sites due to the measured $^1J_{C1\text{-}C2}$ and $^1J_{C3\text{-}C4}$ coupling constants and the 6-OH would make a highly disfavoured 7-membered ring.

Inferring a tridentate binding to D-sorbitol, which is the only reasonable justification of its high binding constant compared to that of D-fructose, only the 5-OH is left for binding, thus giving a 2,3,5-bound complex. A comparison of the measured $^3J_{HH}$ coupling constants with those calculated from the H–C–C–H torsional angles[26] of an MM2 minimised model of the 2,3,5-bound complex is shown in Table 5 and this strongly supports the 5-OH as the third binding site.

We have, from measured NOEs, attempted to determine the approximate orientation of both the side chain and sorbitol

Table 3 $^1J_{CC}$ coupling constants (Hz) of D-sorbitol and the **1·Sorb** complex

	$^1J_{C1\text{-}C2}$	$^1J_{C2\text{-}C3}$	$^1J_{C3\text{-}C4}$	$^1J_{C4\text{-}C5}$	$^1J_{C5\text{-}C6}$
D-Sorbitol[a]	41.4	41.5	41.5	41.5	41.5
1·Sorb[b]	42.9	36.7	40.8	40.4	42.9

[a] In D_2O, values ± 0.1 Hz. Measured by 1D INADEQUATE in natural abundance. [b] In D_2O–CD_3OD (2 : 1 w/w) pH = 8.50, values ± 0.3 Hz. Measured on a sample with (UL)-$^{13}C_6$-sorbitol.

Table 4 Comparison of ^{13}C NMR chemical shifts (ppm) of free D-sorbitol and D-sorbitol part of **1·Sorb**

	Sorbitol[a]	**1·Sorb** complex[b]	Difference
C-1	63.4	64.3	−0.1
C-2	74.2	73.4	−0.8
C-3	70.5	75.0	+4.5
C-4	72.1	67.8	−4.3
C-5	72.1	75.2	+3.1
C-6	64.0	65.0	+1.0

[a] In D_2O–CD_3OD (2 : 1 w/w) pH = 8.50. [b] In D_2O–CD_3OD (2 : 1 w/w) pH = 8.54.

Table 5 Comparison of measured and calculated vicinal J_{HH} coupling constants

		$^3J_{HH}$/Hz		
Molecular fragment	Calculated dihedral angles/°	Haasnoot–Altona[a]	Karplus[b]	Measured values
H2–C2–C3–H3	87.6	0.86	1.4	~0
H3–C3–C4–H4	56.9	4.17	3.1	4.0
H4–C4–C5–H5	161	7.78	9.3	9.3

[a] Calculated from ref. 26 with no β-correction. [b] $7.76\cos^2(\varphi) - 1.1\cos(\varphi) + 1.4$ (ref. 26).

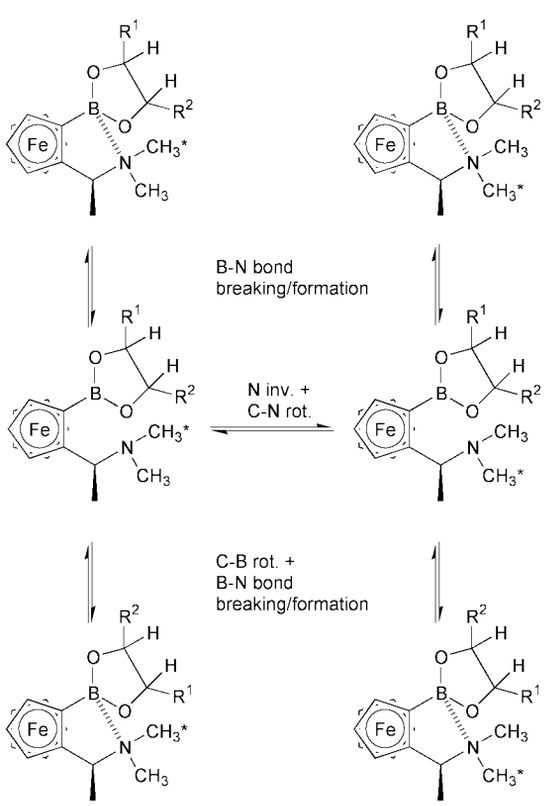
moieties of the **1·Sorb** complex. As shown in Fig. 6 a medium NOE from the C-14 methyl group to H-9 of the ferrocene indicates that the methyl group lies near the plane of the cyclopentadienyl ring of the ferrocene, which is in accordance with our recent findings for similar compounds in the solid state.[13] A strong NOE between H-3 and H-4 supports the deduced 2,3,5-bound structure, as in this structure these protons are fixed in a *gauche* conformation. According to the structure no other vicinal proton pairs should be expected to give a similar strong NOE. This fully agrees with our observations and only the geminal H-6a/b pair gives a matching strong NOE. A weak NOE between the C-15 methyl group to H-5 of the sorbitol chain may indicate a preferred orientation of the sorbitol moiety as drawn in Fig. 6, which implies a short distance between these atoms.

Intramolecular B–N bonding

As we mentioned in the introduction, there are several reasons to question the existence of a B–N interaction in the ferroceneboronic acid (**1**) and its complexes. With the above determination of the structure of the complex **1·Sorb** we have excluded the formation of a B–N bond here, but the question remains whether this interaction may be present in **1** itself and in diol-type complexes.

Some of the evidence reported in this work could indeed be interpreted in favour of a B–N interaction. For example, the coalescence phenomena observed for the N–CH_3-signals of the free ferroceneboronic acid (**1**) (Fig. 3) could be explained by the equilibria depicted in Scheme 1, which are similar to a mechanism proposed by G. Wulff for an analogous *o*-(*N*,*N*-dimethylaminomethyl)benzeneboronic acid.[27] Even for the **1·Sorb** complex, the more complex coalescence phenomenon (Fig. 2) could result from a B–N interaction, thus invalidating the binding of the 5-OH group.

We believe that the explanation for these phenomena is rather one of steric hindrance from both the ferrocene and boronic acid moieties in the process of interchanging the *N*-methyl groups (nitrogen inversion followed by C–N bond rotation) (Scheme 1). In case of *N*,*N*-dimethyl-1-aminoethylferrocene it



Scheme 1

is well known that steric hindrance of the rotation of the C_{Fc}–C bond makes diastereoselective *ortho*-lithiation possible. The (*S*) stereochemistry of the alkylamino group in **1** implies the geometry depicted in Fig. 7, which suggests a restricted rotation around the C–N bond. As mentioned above, the measured NOEs within the **1·Sorb** complex agree with this side chain geometry and, furthermore, the same geometry has recently been determined by us for crystals of uncomplexed **1** and a number of derivatives.[13] For the ferroceneboronic acid (**1**), a strong intramolecular hydrogen bond between the amine and the boronic OH group was observed and in no case did we observe an intramolecular B–N bond.

We have titrated both the ferroceneboronic acid (**1**) and *o*-(*N*,*N*-dimethylaminomethyl)benzeneboronic acid to obtain further insight into these matters. The results from this study are shown in Fig. 8. For *o*-(*N*,*N*-dimethylaminomethyl)benzeneboronic acid we found $pK_1 = 5.2 \pm 0.1$ (lit.[28] $pK_1 = 5.2$) and $pK_2 = 10.7 \pm 0.1$ (lit.[28] $pK_2 = 11.8$) (See Fig. 8, upper curve). These values refer to the equilibrium shown in Scheme 2 and show the species **II** to be present in a pH-window from ~5 to ~11.

The titration curve for the ferroceneboronic acid (**1**) (Fig. 8, lower curve) differs markedly from the former one. From the curve $pK_1 = 8.5 \pm 0.1$ and $pK_2 = 10.7 \pm 0.1$ were determined,

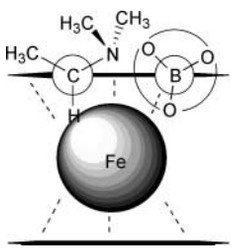


Fig. 7 Schematic representation of steric crowding within **1** and the **1·Sorb** complex.

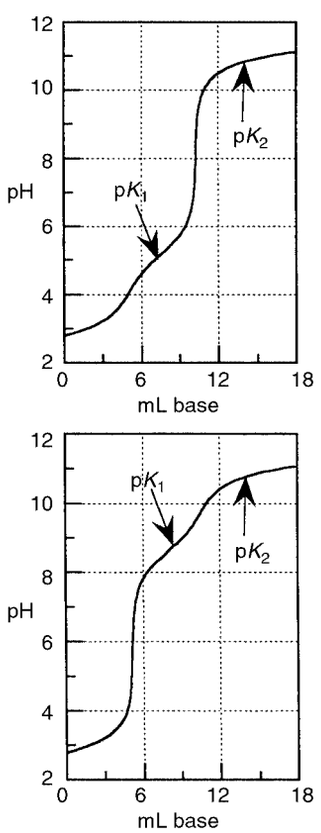
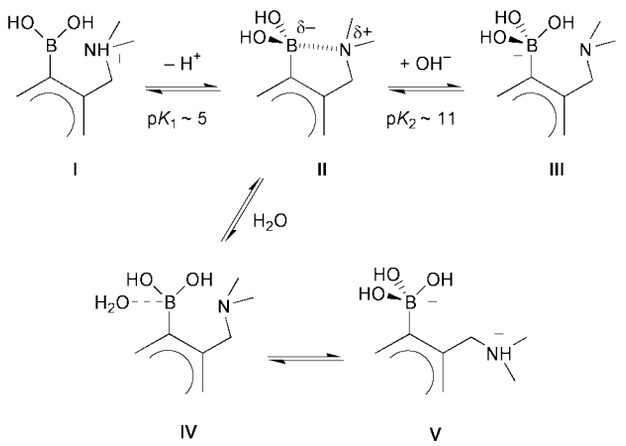


Fig. 8 Titration curves in water of *o*-(*N*,*N*-dimethylaminomethyl)-ferroceneboronic acid (upper) and of (*S*,*S*)-2-(*N*,*N*-dimethyl-1-aminoethyl)ferroceneboronic acid (**1**) (lower).

which implies that K_1 is three orders of magnitude less than for *o*-(*N*,*N*-dimethylaminomethyl)benzeneboronic acid. Our only explanation for this observation is that a B–N bound species such as **II** (Scheme 2) does not exist. When we do not have



Scheme 2

a strong B–N interaction the aminoboronic acid will behave as a normal "amino acid" with the zwitterionic form **V** present as the major species between the two p*K* values.

^{11}B NMR

We have obtained the ^{11}B NMR spectra of the ferroceneboronic acid (**1**) at varying pH and under the same conditions as the above-described NMR experiments (D_2O–CD_3OD 2 : 1). Fig. 4 (entries a, b and c) shows the spectra at pH = 7.4 to pH > 12. At the lower pH (entry c) we observe a chemical shift of 34 ppm corresponding to the free trigonal planar boronic acid. By adding base we find an intermediate situation (entry b) where we measure an approximate pH of 12 in the water–methanol mixture.[29] At this pH we observe a chemical shift of 26 ppm, corresponding to an increased shielding of 8 ppm. By adding more base the peak shifts to its lowest value of 13 ppm (entry a) corresponding to an increased shielding of ~20 ppm. This shielding corresponds to that found for benzene boronic acid when going from the free acid to the tetrahedral anionic boronate[30,31] and, approximately, to our findings for the unsubstituted ferroceneboronic acid which was studied here only for this comparison (Fig. 5, entries a, b and c).

For the solution corresponding to Fig. 4, entry b we observe the slow crystallisation of yellow needles. Under these conditions we believe the major species present to be the neutral form of the ferroceneboronic acid (**1**) (either **II**, **IV**, **V**, Scheme 2). We have determined the structure of the crystals to be identical to those obtained upon crystallisation of **1** from diethyl ether. This structure does not show an intramolecular B–N bond, but instead an intramolecular hydrogen bond between the B–OH group and the nitrogen atom.[13]

The ^{11}B NMR spectra of the **1·Sorb** complex are supportive of the conclusions drawn from the ^{1}H and ^{13}C NMR studies. From Fig. 4 (entries d to g) we can see how, from having uncomplexed boronic acid **1** at pH = 3.4 (entry d), we go to a mixture of **1** and **1·Sorb** complex at pH = 7.4 in accordance with the ^{1}H NMR experiments. The ratio of complex and uncomplexed **1** at this pH agrees with the one obtained from the ^{1}H NMR experiment (see "binding constant" discussion below). At higher pH we observe only the signal from the **1·Sorb** complex. The enhanced shielding of 23 ppm, compared to the uncomplexed free boronic acid form, agrees with a tetrahedral boronate and is further in agreement with the shielding found in the tridentate β-D-fructofuranose 2,3,6-tri-*O*-(*p*-phenylalanylorthoboronate) compared to the free *p*-boryl-phenylalanine.[10] In case of a B–N bound bidentate structure a shielding of only 10–15 should have been expected.[27,32] Finally, we did two experiments where we added an excess (5 equiv.) of pinacol and 2,2-dimethylpropane-1,3-diol, respectively to ferroceneboronic acid (**1**) under alkaline conditions. The spectra are shown in Fig. 5, entries d and e. As seen from Fig. 5, entry d, the five-membered pinacol ester forms, having chemical shift of 15 ppm. Fig. 5, entry e shows that the six-membered (2,2-dimethylpropane-1,3-diyl)boronate is formed in smaller amounts but has increased shielding compared to the five-membered ester. This is in accordance with the observations for the corresponding borate esters. That the broad absorption at 11 ppm in Fig. 5, entry e does not correspond to the absorption of the non-bound tetrahedral boronate can be deduced from the fact that only for the diol-bound **1** is the equilibrium slow enough to give separate ^{11}B NMR absorptions (compare with Fig. 4, entries a–c). It should be noted that this equilibrium seems to be slower in the case of ferroceneboronic acid itself, which lacks the *ortho*-amino substituent.

The chemical shift of 11 ppm for the six-membered neopentylboronate corresponds to the value found for the **1·Sorb** complex and, although no simple comparison can be made, we interpret this result as supportive evidence for the **1·Sorb** complex holding a six-membered ring, in accordance with our conclusion of a 2,3,5-complexed sorbitol.

Binding constant

The binding constant of the 1 : 1 complex has been estimated from the ^{1}H NMR spectrum at pH = 7.4 and 0 °C. In contrast to the situation at pH = 8.6 as described above, signals from unbound sorbitol and free boronic acid **1** appeared at pH = 7.4. By integration of signals from one *N*-methyl group of the free boronic acid (2.42 ppm) and of the complex (2.30 ppm) the ratio between free and bound **1** was estimated to be 1 : 2.2. As the initial concentrations of **1** and D-sorbitol were both 48 mM the binding constant can be calculated to be $K = 147$ M^{-1}. This estimate corresponds very well with the earlier electrochemically-determined value of 110 ± 10 M^{-1} at pH 7.0.[3] Recently an electrochemical study of a closely-related ferroceneboronic acid (without the aminoalkyl substituent) appeared.[33] The authors found a significantly lower value of 11 M^{-1} (pH = 7.6) for the binding constant of the non-oxidised form, but reported a substantially higher value for the oxidised form, which is also the case for ferroceneboronic acid **1**. These results lead again to speculations on the role of the amino substituent with respect to stabilisation of the non-oxidised complex.

Coalescence phenomena

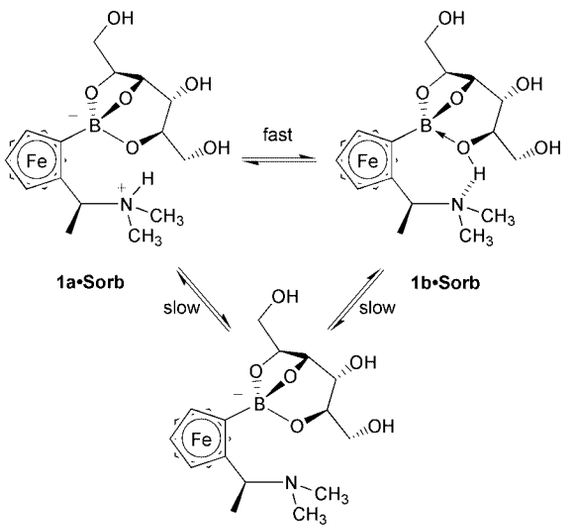
The coalescence phenomena depicted in Fig. 2 and 3 need some further discussion. As mentioned above, we have found no evidence which can be ascribed to an intramolecular B–N interaction in either the ferroceneboronic acid (**1**) alone or its sorbitol complex. For the non-complexed boronic acid we have argued that steric interactions may cause an energy barrier to the exchange of *N*-methyl groups. From the data in Figs. 3A and 3B values of $\Delta G^{\ddagger}_c = 59.4$ and 54.5 kJ mol^{-1}, respectively, can be calculated. However, if one considers a B–N interaction, and therefore the processes in Scheme 1, a $\Delta G^{\ddagger}_c = 61.3$ kJ mol^{-1} for B–N bond breaking is found in the water–MeOH buffer [k_c for this process would be k_c(obs)/2].[27] The calculated value is more than 20 kJ mol^{-1} higher than that found by Wulff *et al.*[27] and others[34,35] for the *ortho*-substituted benzene analogue.

The situation within the **1·Sorb** complex is surely more complex (Fig. 2). Here no simple coalescence can be defined and upon heating at pH = 8.6 we observe two resulting broad absorptions from the *N*-methyl groups at 2.4 and 2.6 ppm in a ratio of approximately 1 : 2. After some consideration our conclusion on this observation was that we, at this intermediate pH, had to be encountering two structurally very similar

complexes present in a slow internal equilibrium. As, by heating the sample, we observed no large changes in the absorptions from the sorbitol part, we concluded that the two complexes present could only differ around the amine functionality. The difference in line width led to the conclusion that our observations could be caused by two distinct complexes, one of which contained a protonated amine and one the neutral amine.

To test this we repeated the experiment at a higher pH (Fig. 2B). This experiment showed that, upon heating the former, two broad absorptions now collapsed into a single sharp absorption at 2.54 ppm.

Our explanation of these observations is summarised in Scheme 3. At intermediate pH we believe that we have a slow



Scheme 3

equilibrium between the overall neutral species **1a·Sorb** and **1b·Sorb**, on the one hand, and the anionic **1c·Sorb** complex on the other hand. That the coordinative complex **1b·Sorb** is in a fast equilibrium with **1a·Sorb** is reasonable, compared to our observations of a strong intramolecular hydrogen bond in the solid state of the free boronic acid. As the broad absorptions at pH = 8.6 are only separated by 0.2 ppm, we conclude the coordinative complex **1b·Sorb** to be the major species in the fast **1a·Sorb/1b·Sorb** equilibrium, as the resonances of the *N*-methyl groups of a protonated amine (as in **1a·Sorb**) would be expected to appear at lower field. We have not, for this work, attempted to assign the two broad resonances at 2.4 and 2.6 ppm to either of the **1a·Sorb/1b·Sorb** or **1c·Sorb** complexes, but further NMR-titrations may clarify this point. Furthermore, extended investigations of the kinetics and thermodynamics of the **1a·Sorb/1b·Sorb** equilibrium are beyond the scope of this work.

From Scheme 3 it is clear that the anionic 2,3,5-bound **1c·Sorb** will be the sole species present at high pH. In full accordance with our observations at high pH (Fig. 2B) this should simplify the NMR spectrum resulting in a single *N*-methyl absorption at high temperature.

Conclusions

We have above presented an extensive investigation on what, initially, seemed to be a very simple 1 : 1 ferroceneboronic acid–sorbitol complex. Encouraged by the clear "easy to assign" ^{1}H NMR spectrum we started our investigation leading to this fascinating insight into new aspects of boronic acid–polyol interactions. We have provided strong evidence for a 2,3,5-bound sorbitol complex, thus excluding an intramolecular B–N interaction in this complex. Further investigations, including our crystallographic studies in the following paper,[13] have shown this type of interaction to be highly questionable within **1** and close derivatives, probably due to geometrical and steric constraints. Our titration of **1** provides very strong evidence that a B–N interaction is not present in this 1,2-substituted ferrocene derivative. Therefore compound **1** will need to be modified to some extent before it shows useful properties as, *e.g.*, a glucose sensor with a working pH of 7.4.

In an earlier work we presented the structure of a complex between an anthracene-based host and a glucofuranose including both a B–N bound and a zwitterionic motif. In the present work we have further substantiated the importance of both coordinative- (**IV**, Scheme 2) and zwitterionic species (**V**, Scheme 2) when dealing with sensor molecules containing both amino and boronic acid groups. Very careful design of future carbohydrate sensors is therefore needed in order to gain the benefits of the remarkable B–N bonding originally demonstrated by Wulff.[28]

Experimental

General

^{1}H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively. COSY and C–H correlated spectra were recorded at 400 MHz. HSQC and NOESY experiments were performed at 600 MHz. Chemical shifts are reported in ppm and for all spectra the data are referenced internally to the residual CD_2HOD peak at 3.30 ppm (^{1}H) and 49.0 ppm (^{13}C). ^{11}B NMR spectra were recorded in 5 mm quartz tubes (Wilmad 507-pp) and referenced externally to $NaBF_4$ in D_2O, $\delta = +2.3$ ppm; *i.e.*, δ ($BF_3{\cdot}Et_2O$) = 0.0 ppm).[36] The term "pH" has, throughout the paper, been used as equal to "pD".

Materials

Ferroceneboronic acid was made by the method of Epton *et al.*[37] (*S*,*S*)-2-(*N*,*N*-Dimethyl-1-aminoethyl)ferroceneboronic acid (**1**) was synthesised by an analogous method to that of Silva *et al.*[14] as described elsewhere.[13] Deuterated solvents and reagents were purchased from Cambridge Isotope Laboratory.

pK_a determinations

Titrations were performed as triplicate determinations at 25 °C on an automated Metrohm Titrino titrator equipped with a thermostated reaction chamber and a Metrohm 6.0238.000 combined glass electrode.

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