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Structures of carbohydrate–boronic acid complexes determined by NMR and molecular modelling in aqueous alkaline media

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Received 20th October 2003, Accepted 26th February 2004
First published as an Advance Article on the web 19th April 2004

The structures of thermodynamically stable aromatic boronic acid : cyclic carbohydrate chelates in aqueous alkaline media have been studied using ^{1}H NMR spectroscopy and molecular modelling. It is found that interacting saccharides must necessarily possess a synperiplanar diol functionality for complexation to occur. While this is possible for furanose structures which tend to have a puckered planar geometry, for pyranose forms it is postulated that bis-complexation occurs with twist conformers of the pyranose ring, providing the ring has the requisite 1,2 : 3,4 polyol stereochemistry; specifically axial,equatorial : equatorial,axial or equatorial,axial : axial,equatorial orientations. In this respect it is possible to be predictive with regard to individual carbohydrate boronic acid interactions and to give reasonably comprehensive structural assignments to complexed components. In this paper twenty four polyhydroxy compounds have been screened using ^{1}H NMR to monitor complexation along with computational techniques on a model system to substantiate proposed structures. It has been found that all of these materials interact with aromatic mono boronic acids as expected and structures for the resulting chelates are proposed.

Introduction

Aromatic boronic acids have gained attention in recent years as specific sensor molecules due to an ability to recognise carbohydrate substrates, particularly in aqueous environments.[1,2] The main thrust of the work has been concerned with perfecting aqueous sensing techniques for drug delivery,[3] electrophoresis,[4,5] molecular transportation across liquid membranes,[6–9] and chromatography. In most cases the reports centre on the use of physical measurement techniques to obtain the association constants of boronic acid derivatives with a range of structurally diverse mono, di and tri saccharides, the most relevant being a technique developed by Wang *et al.* which uses the fluorescent reporter Alizarin Red S to determine boronate ester stability.[10] In more recent years research groups have concentrated their efforts on increasing these stability constants with physiologically important carbohydrates by constructing elaborate diboronic acid molecules comprising appropriate spacer units, or by incorporating adjacent amine groups to promote tighter binding *via* photoinduced electron transfer.[11–17] There has however been a paucity of research papers dedicated to the elucidation of the complexed structures, or an attempt at establishing ground rules with respect to regio and stereochemical requirements. In its broadest sense the mechanism of boronic acid complexation with saccharides, *e.g.* glucose, can be regarded as a reversible and rapid formation of water stable covalent bonds between the two substrates producing an equilibrium mixture of complexed materials (Fig. 1).

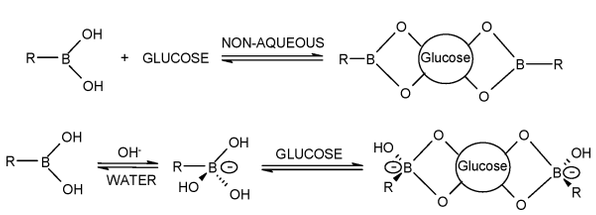

Fig. 1 Schematic representation of aromatic boronic acid interactions with saccharides in non-aaqueous and aqueous alkaline media.

A survey of the literature indicates that the precise structure of the complexes involved is subject to diverse opinion, with some groups favouring the formation of glucopyranose boronate esters[1,2] and others inclined towards glucofuranose complexation.[18–21] An investigation by Norrild and Eggert however has supplied convincing factual evidence. Using ^{1}H and ^{13}C NMR spectroscopy, with the application of $^{1}J_{C\text{-}C}$ coupling constants as a structural probe, they have shown that in all cases the furanose form of glucose is bound to *para*-tolylboronic acid up to stoichiometric ratios of 2 : 1.[22] Furthermore under neutral non-aqueous conditions binding occurs at the α-1,2 : 3,5 positions (Fig. 2a), and under aqueous alkaline conditions at the α-1,2 : 3,5,6 positions forming *endo* and *exo* diastereomers (Fig. 2b and c).

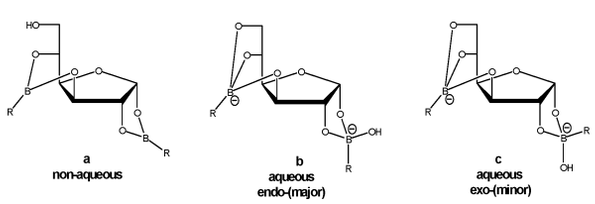

Fig. 2 The 3 assigned structures to α-glucose boronate esters under non-aqueous and aqueous alkaline conditions.[22]

On the face of it this is not surprising. Boron–oxygen bond lengths are known to be relatively short, due to dative pπ–pπ overlap of an empty boron orbital with an oxygen lone pair in trigonal molecules, and the covalent–ionic resonances associated with a large electronegativity difference in tetrahedral molecules. Therefore in the absence of rotational freedom about the C–C bonds, the formation of thermodynamically stable boronate ester complexes will require the interacting 1,2 diol groups to have small dihedral angles in a synperiplanar arrangement with a concomitant easing of ring strain. In the glucopyranose $^{4}C_{1}$ chair conformation all the hydroxyls are equatorial with dihedral angles of ±55° and therefore synclinal with respect to each other. However rearrangement to the furanose form gives the required diol coplanarity at the 1,2 positions for the α anomer (Fig. 3). The significance of this is apparent when we consider that α-glucofuranose is present in water at only 0.14%.[23]

In a similar study using identical 2D NMR techniques, the same authors have reported on the structural assignments of boronic acid complexation with fructose.[24] At a boronic

DOI: 10.1039/b312760e

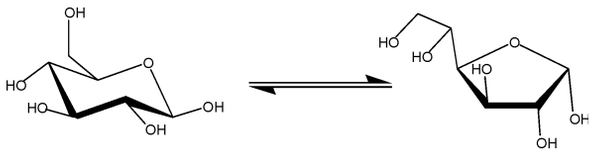

Fig. 3 Interconverting pyranose and furanose forms of α-glucose.

acid : fructose ratio of 1 : 1 in aqueous alkaline solution, monoboronate esters are formed with β-fructofuranose. The major species is the 2,3,6 tridentate complex (Fig. 4a), while the 2,3 *endo* and *exo* isomers are also present (Fig. 4b and c).

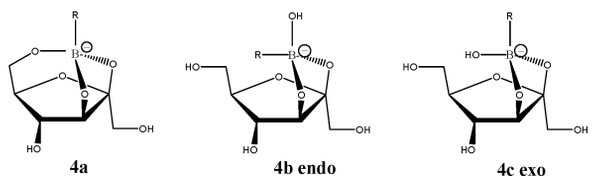

Fig. 4 Structures assigned to β-fructose boronate esters at equimolar boronic acid : carbohydrate concentrations.[24]

Considering the rationale for glucose complexation this is in good agreement with diol coplanarity. However it will be noticed that fructofuranose lacks a polyol set-up compatible with diboronate ester complexation. As such at higher boronic acid concentrations, *i.e.* ratios of 2 : 1 and 4 : 1, four new complexes are observed which become the predominant species. These have been identified and assigned as *endo* and *exo* diboronate ester isomers of β-fructopyranose (Fig. 5a–d).

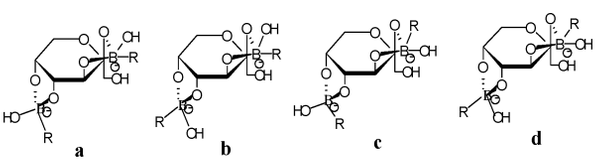

Fig. 5 Structural assignment of additional β-fructose boronate esters formed at higher boronic acid : carbohydrate ratios.[24]

β-Fructopyranose has a $^1C_4$ chair conformation with the 4 secondary hydroxyls in a synclinal arrangement. Under these conditions we would not expect to see boronic acid interaction and therefore to explain this observation it is pertinent to look at the boat/twist intermediate between the two chair conformers. Due to the original axial,equatorial : equatorial,axial orientations of the 2,3 : 4,5 positions respectively, the twist conformer gives a significant reduction in the pyranose inter-hydroxyl torsional angles as the geometries become more eclipsed. Although the twist conformer is energetically unfavourable, the energy pay-off could be attained from the enthalpy of additional complexation. We have subsequently found one literature paper by van den Berg *et al.* demonstrating that boronate esters do form with 1,2 axial : equatorial pyranose structures but not with 1,2 diequatorial pyranoses.[25] Further convincing evidence for this idea comes from the reported $^1J_{C\text{-}C}$ coupling constants of the complexes formed. It is known that the C–O torsional angles in O–C–C–O fragments, by means of oxygen lone pair orientation, are the most important parameters determining the size of $^1J_{C\text{-}C}$ in these systems giving a maximum for *anti* and a minimum for eclipsed configurations.[24,26] The experimental values are considerably reduced for the C2–C3, and to a lesser extent for the C4–C5 positions, with a corresponding increase at the C3–C4 position in the bis complexed species when compared to uncomplexed fructopyranose. This is consistent with the molecular rearrangement from a staggered *syn* to an eclipsed *syn* orientation for the former 2 positions, and a change to a staggered *anti* geometry for the C3–C4 accompanying a chair to twist transformation (Fig. 6).

A consideration of the foregoing therefore enabled the proposal of a plausible theoretical evaluation of the fundamentals

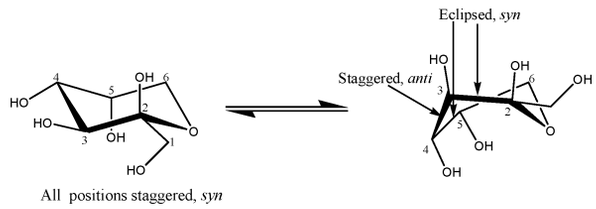

Fig. 6 Interconverting chair and twist conformations of β-fructopyranose showing the changes in orientation which affect NMR coupling constants.

of boronic acid carbohydrate complexation in aqueous alkaline solution. By establishing the basic principles it is considered that for any carbohydrate molecule we can not only predict whether it would form mono/bis complexes with aromatic mono boronic acids, but also give a reasonably comprehensive structural assignment of components. For substantiation we have recently screened twenty four commercial polyhydroxy materials of diverse structures, using ^{1}H NMR analysis to monitor complexation. It has been found that all of these compounds interact with an aromatic boronic acid (*meta*-nitrophenylboronic acid, *m*NPBA) as expected. In addition the molecular modelling of galactose with phenylboronic acid (PBA) is consistent with these results and is reported herein.

NMR experiments

The ^{1}H NMR spectra were recorded on a Bruker 360 MHz spectrometer in D_2O and referenced internally to sodium 3-(trimethylsilyl)-1-propane sulfonic acid TSP. The solutions were made up at 10^{-2} M in carbohydrate with pH adjustment to 9.5 using NaOD, and at 0.1 M ionic strength using potassium nitrate. The spectra were recorded at ambient temperature. Chemical shifts are given in ppm.

Materials

All of the materials were used as obtained from Sigma Chemicals Ltd. The 24 molecules screened were:

cis-1,2-cyclopentanediol **1**; *trans*-1,2-cyclopentanediol **2**; *cis*-1,2-cyclohexanediol **3**; *trans*-1,2-cyclohexanediol **4**; glucose **5**; 1-*O*-α-methylglucopyranose **6**; 1-*O*-β-methylglucopyranose **7**; 3-*O*-methylglucopyranose **8**; galactose **9**; 1-*O*-α-methylgalactopyranose **10**; 1-*O*-β-methylgalactopyranose **11**; mannose **12**; arabinose **13**; xylose **14**; ribose **15**; fructose **16**; sorbose **17**; tagatose **18**; cellobiose **19**; maltose **20**; gentiobiose **21**; melibiose **22**; sucrose **23**; palatinose **24**.

Molecular modelling

Semi-empirical quantum chemistry software (MOPAC–AM1)[27] was used to calculate the heats of formation of the complexes. Calculations were performed for different phenylboronic acids complexed to various conformations of furanose and pyranose rings of α-galactose. All visualisation was rendered using Accelrys software InsightII.[28] Validation calculations performed on test systems from the CSD[29] showed that the geometry around the boronic acid moiety was well reproduced.

Results and discussion

Four cycloalkane-1,2-diols were screened with *m*NPBA initially as a means of obtaining a simple working model. The ^{1}H NMR data is given in Table 1.

cis-cyclopentane-1,2-diol (**1**) forms a stable complex with the boronic acid as expected due to its synperiplanar dihydroxy moiety giving two complexed signals. At the experimental pH (9.5) the *m*NPBA (pK_a 6.9) will be tetrahedral providing additional stereocenters and therefore the two complexes can be assigned as the *endo* and *exo* isomers shown in Fig. 7.

Table 1 Cycloalkane-1,2-diol : *m*NPBA complexes in a 1 : 1 ratio

	Substrate	C-1 substrate[a]	C-1 complexed[a]	Ratio	Chem. shift increment	Degree of complex[n]
	1	4.02	4.33 : 4.38	65 : 35[b]	0.31–0.36	50%[b]
	2	4.01	[c]	—	—	—
	3	3.80	[c]	—	—	—
	4	3.38	[c]	—	—	—

[a] ^{1}H NMR ppm. [b] Approximate values. [c] No complex formed.

Table 2 Aldose : *m*NPBA complexes (**5**–**15**)

Ald.	C-1 substrate[a]	BA : substrate ratio	C-1 complexed[a]	Ratio	Chem shift increment	Degree of complex[n]
5	5.23	1 : 1	6.25:5.97:5.90;5.84	41:14:34:11	0.61–1.02	55%
5	5.23	2 : 1	6.25 : 5.97 : 5.90 : 5.84	[b]	0.61–1.02	70%
6	4.83	2 : 1	[c]	—	—	—
7	4.38	2 : 1	[c]	—	—	—
8	5.22	1 : 1	5.86 : 5.79	73 : 27	0.57–0.64	59%
8	5.22	2 : 1	5.86 : 5.79	73 : 27	0.57–0.64	69%
9	5.27	1 : 1	5.82	100	0.55	65%
9	5.27	2 : 1	5.82	100	0.55	78%
9	5.27	5 : 1	5.82 : 5.65 : 5.55 : 5.50 : 5.40	58 : 22 : 8 : 9 : 3	0.13–0.55	97%
10	4.85	2 : 1	[c]	—	—	—
11	4.33	2 : 1	[c]	—	—	—
12	5.19	1 : 1	5.51 : 5.34	84 : 16	0.15–0.32	24%
12	5.19	2 : 1	5.51 : 5.34	73 : 27	0.15–0.32	35%
13	5.24	1 : 1	5.92 : 5.84	14 : 86	0.60–0.68	74%
13	5.24	2 : 1	5.92 : 5.84	12 : 88	0.60–0.68	86%
14	5.20	1 : 1	6.00 : 5.92 : 5.85	9 : 70 : 21	0.65–0.80	68%
14	5.20	2 : 1	6.00 : 5.92 : 5.85	15 : 65 : 20	0.65–0.80	83%
15	4.94	1 : 1	5.77	100	0.83	65%
15	4.94	2 : 1	5.77	100	0.83	81%

[a] ^{1}H NMR ppm, [b] ratios difficult to measure due to broadening of bands, [c] no complex formed .

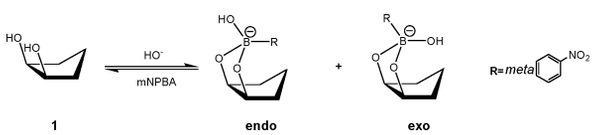

Fig. 7 *endo* and *exo* Isomers of *cis*-1,2-cyclopentanediol boronate esters; R = *meta*-nitrophenylboronic acid (*m*NPBA) is also shown.

The *trans* isomer (**2**) has an anticlinal diol and as expected complexation is not observed. It was found that neither of the two cyclohexane-1,2-diols form complexes with *m*NPBA which agrees with previously published stability data.[30] For the *cis* isomer (**3**), which has an axial : equatorial dihydroxy, trans-formation through the boat/twist conformers should produce an eclipsed *syn* diol. However, by flipping from one chair conformation to the other, conformational enantiomers are obtained and hence interconversion will be too rapid for complexation to occur. The *trans* isomer (**4**) is diequatorial and cannot acquire diol coplanarity for any conformer.

In total eleven aldoses were screened comprising eight aldohexoses (**5**–**12**) and three aldopentoses (**13**–**15**), the ^{1}H NMR data for the starting material and complexed anomeric protons is presented in Table 2.

The boronic acid complexation of glucose (**5**) has been discussed earlier and little further need be added here. The four complexed anomeric protons can be ascribed to the mono 1,2 and diboronate esters of α-glucofuranose in their *endo* and *exo* forms (*cf* Fig. 2). The relatively large chemical shift increments (0.61–1.02 ppm) are assumed to be indications of furanose complexation in a similar manner to ^{13}C shifts.[24,25,31–36] Compounds **6** and **7** are glucopyranosides methylated α and β respectively at the anomeric site. Substitution at this position means that the structures cannot rearrange to the furanose form and as the remaining hydroxyls are all equatorial, diol coplanarity is geometrically impossible in any of the chair or boat/twist conformations. Complexation with *m*NPBA is therefore not observed. Methylation at other ring positions gives a different outcome however, for example 3-*O*-methyl-glucopyranoside (**8**) can convert to the furanose form yielding a synperiplanar diol functionality at the 1,2 position for the α anomer. As such two major complexed anomeric proton signals are seen which correspond to monoboronate *endo* and *exo* isomer forms (again *cf* Fig. 2). Here diboronate ester formation is precluded due to methyl substitution on the 3-hydroxyl, although minor complexed signals at 5.82 and 5.64 might suggest 5,6 complexation or α-pyranose complexation.

Galactose (**9**), both at 1 : 1 and 2 : 1 *m*NPBA ratios, has a fairly simple profile in that only one major complexed anomeric proton is observed in the NMR spectra at 5.82 ppm showing a 0.55 ppm chemical shift increment from the uncomplexed material. The magnitude of this shift suggests pyranose to furanose rearrangement and complexation is envisaged to be tridentate at the α-1,2,5 positions, di-complexation being unlikely as the 3 and 5,6 hydroxyls are on opposite faces of the ring. This structure, which has been reported by van den Berg and co-workers using ^{13}C data,[25] is possible when the 5-hydroxyl is oriented gg and is analogous to the fructofuranose complex. A minor signal at 5.89 ppm comprises 6% of the total hydrogen intensity and is thought to be the *exo* 1,2 mono boronate ester, the *endo* isomer being sterically inhibited by the 5,6 dihydroxy unit.

It was further reasoned that α-galactopyranose has an analogous structure to β-fructopyranose in that the 1,2 : 3,4 positions have axial,equatorial : equatorial,axial orientations. Therefore the boronic acid ratio was increased to 5 : 1 in an attempt to induce diboronate ester formation with the twist conformer in the expectation that four *endo*/*exo* isomers would be obtained in a similar fashion to fructose. This appears to have been achieved with 4 new signals appearing at 5.65; 5.55; 5.50 and 5.40 ppm. Furthermore these new complexed anomeric protons have broader bands and smaller chemical shift increases of the

order 0.13–0.38 ppm which would be expected for pyranose complexation. The proposed structures for these complexes are shown in Fig. 8.

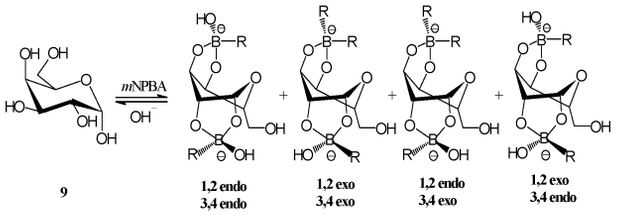

Fig. 8 Proposed structures of α-galactopyranose boronate esters at high boronic acid : carbohydrate ratios (discussed in the modelling section).

Further to this a series of molecular modelling calculations also predicts structures in accord with this hypothesis. These are outlined later within this discussion.

The α and β methylated galactopyranosides, compounds **10** and **11** respectively, both failed to show complexation with *m*NPBA. In both these materials the 3,4 inter-hydroxyl torsional angles will decrease in the twist form as in unmethylated galactopyranose, but it is well documented that the more reactive anomeric site needs to be free for complexation as the primary site of boronate ester formation.[37]

From the NMR data in Table 2 the evidence suggests that mannose (**12**) does not form boronic acid complexes in its furanose form. A close look at this molecule reveals that β-mannofuranose will have all five hydroxy groups on the same face of the five membered ring which will have an unstable configuration. However it is known from the literature that mannose does interact with boronic acids and that the 6-hydroxyl appears to be involved in the complexation.[38,39] In the pyranose twist conformation β-mannopyranose has an eclipsed *syn* 1,2-diol and complexation here can be stabilised by tridentate co-ordination with the 6 position as shown in Fig. 9. The two complexed anomeric proton signals at 5.51 and 5.34 ppm are therefore probably tridentate and bidentate structures. Again the broad signals obtained combined with the small chemical shift differences, are indications of pyranose complexation.

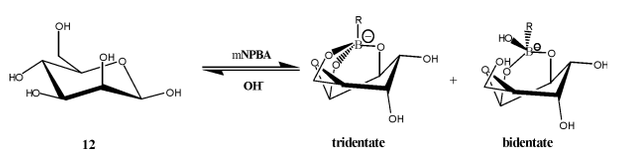

Fig. 9 Probable structures of β-mannopyranose boronate esters as the 1,2,6 tridentate and 1,2 bidentate twist isomers.

The first aldopentose arabinose (**13**), has an analogous structure to galactose (**9**) with the exception of the 6-hydroxymethyl group. It is therefore not surprising that interaction with *m*NPBA follows a similar pattern. One major complex is observed at 5.84 ppm with a minor species at 5.92 ppm. Again complexation is occurring with the α-1,2-arabinofuranose isomer and one would expect this to be the tridentate complex analogous to the β-fructofuranose complex in Fig. 4a. It is possible that the signal at 5.92 ppm is the bidentate 1,2 *endo* stereoisomer with the *exo* form appearing as a shoulder on the 5.84 ppm doublet. The three structures are shown in Fig. 10.

Xylose (**14**) is structurally similar to glucose (**5**), again with the exception of the 6-hydroxymethyl group. Here there are two major complexed anomeric protons with chemical shift differences of the order 0.65–0.72 ppm indicating the furanose structure as expected. From the approximately 3.5 : 1 ratio these are thought to be the *endo* and *exo* diastereomers of α-1,2 complexed xylofuranose comparable to glucose. Due to the additional rotational freedom of the 5-hydroxyl, this is unlikely to take part in bidentate diboronate ester complexation. However, tridentate co-ordination is possible for β-xylofuranose in the 1,3,5 positions explaining the third complex. Evidence for this comes from ^{11}B data from a previous study,[25] although the authors assign this to 3,5 complexation the chemical shift is in the region for a 1,3,5 tridentate complex.

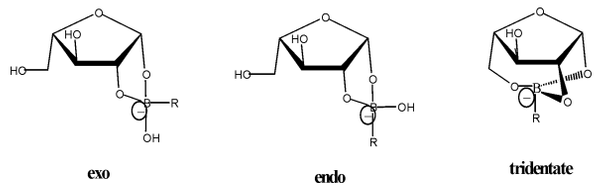

Fig. 10 The 3 likely structures of α-arabinofuranose boronate esters as the *exo* and *endo* 1,2 diastereomers and the 1,2,5 tridentate complex.

The final monoaldose screened was ribose (**15**). Only one complex is observed with an anomeric chemical shift increase of 0.83 ppm. This suggests ribofuranose complexation without the presence of stereoisomers. For α-ribofuranose the 1,2,3 hydroxyls are all on the same face of the ring meaning that a tridentate monoboronate ester is the most likely structure (Fig. 11). Although α-ribopyranose has its 1,2 : 3,4 positions axial,equatorial : axial,equatorial, diboronate complexation cannot occur for steric reasons in the twist conformer as all four hydroxyl groups will be on the same face of the pyranose ring.

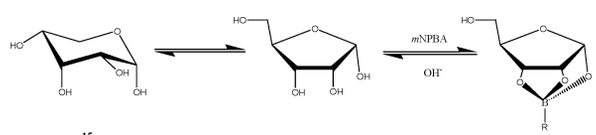

Fig. 11 Proposed 1,2,3 tridentate structure for α-ribose boronate ester in its furanose form.

Fructose (**16**) has already been dealt with comprehensively. Sorbose (**17**) is the 5-epimer of fructose having a similar $^1C_4$ chair conformation. This material also forms complexes with *m*NPBA, but without the assistance of an anomeric proton for elucidation it is difficult to be precise on the nature of interaction. Theoretically at any boronic acid : sorbose ratio we would expect *endo* and *exo* monoboronate esters of β-sorbofuranose. As the 6-hydroxyl is on the opposite face of the furanose ring in this molecule tridentate complexation is not possible, but for the α anomer 2,4,6 tridentate complexation would be expected (Fig. 12). In the pyranose twist conformation, again diboronate esters cannot form due to the equatorial nature of the 3,4 and 5 hydroxy groups.

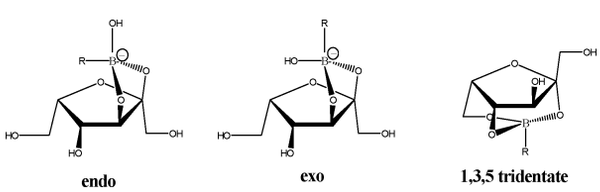

Fig. 12 Proposed structures for β-sorbofuranose as the *endo* and *exo* 2,3 bidentate and the 2,3,5 tridentate boronate esters.

Tagatose (**18**) has a $^4C_1$ chair conformation with the 3,4 and 5 positions being axial, equatorial and equatorial. As such β-tagatofuranose can attain a synperiplanar diol at the 2,3 positions to form a monoboronate ester with probable tridentate nature with respect to the 6 hydroxyl group. Again there does not appear to be any advantage in pyranose twist complexation as the molecule does not have the correct polyhydroxy set-up compatible with bis complexation.

In all six disaccharides were examined and the NMR data for the four dialdose starting materials and complexes is presented in Table 3. Cellobiose (**19**) is a β-1,4-diglucose while maltose (**20**) is its α counterpart. In both these molecules the 1,4 linkage

Table 3 Disaccharide : mNPBA complexes (**19**–**24**)

Substrate	C-1 substrate[a]	BA : substrate ratio	C-1 complexed[a]	Ratio	Chem shift increment	Degree of complex[n]
19	5.23	1 : 1	5.55	100	0.32	6.5%
19	5.23	2 : 1	5.55	100	0.32	8%
20	5.24 : 5.42	1 : 1	5.55	100	0.13–0.31	5%
20	5.24 : 5.42	2 : 1	5.55	100	0.13–0.31	8%
21	5.24	1 : 1	5.90 : 5.84	75 : 25	0.60–0.66	51.5%
21	5.24	2 : 1	5.90 : 5.84	76 : 24	0.60–0.66	64%
22	5.25	1 : 1	5.87 : 5.83	91 : 9	0.58–0.62	63%
22	5.25	2 : 1	5.87 : 5.83	92: 8	0.58–0.62	81.5%

[a] ^{1}H NMR ppm.

means that neither ring can rearrange to the furanose form ruling out the possibility of a relatively stable synperiplanar diol. However for the ring containing the free anomeric hydroxyl the pyranose twist conformer of the α anomer does possess a coplanar diol function although this will be of a transient nature. As such a small degree of complexation is observed for these two disaccharides with a typically small chemical shift increment of approximately 0.3 ppm. Only one complex is seen and on steric grounds this is thought to be the *exo* isomer. The likely structure for cellobiose is shown in Fig. 13 and it can be speculated that maltose will be analogous.

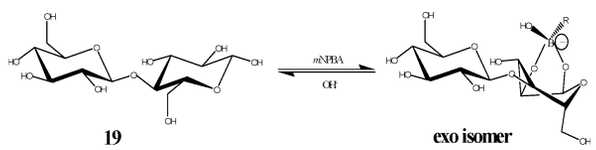

Fig. 13 The expected interaction of cellobiose with aromatic boronic acids. This results in moderate ester formation with the twist conformer at the anomerically labile glucose ring.

Gentiobiose (**21**) and melibiose (**22**) are β-1,6 and α-1,6 linked disaccharides respectively and it can be seen from Table 3 that they afford a much higher degree of complexation with *m*NPBA than the α and β 1,4 counterparts mentioned previously. With these two molecules transformation of one ring (6 substituted) to the furanose form is possible providing a synperiplanar diol moiety for the α anomers. Therefore 1,2 monoboronate esters as *endo* and *exo* diastereomers would be expected and the two complexed anomeric protons in each case coupled with increased chemical shifts between 0.58–0.66 ppm are consistent with this idea. The α linkage for melibiose means that the two rings are not planar with respect to each other which possibly explains the higher degree of *endo* formation for steric reasons. The reaction sequence for gentiobiose complexation is given in Fig. 14, and that for melibiose may be regarded similarly.

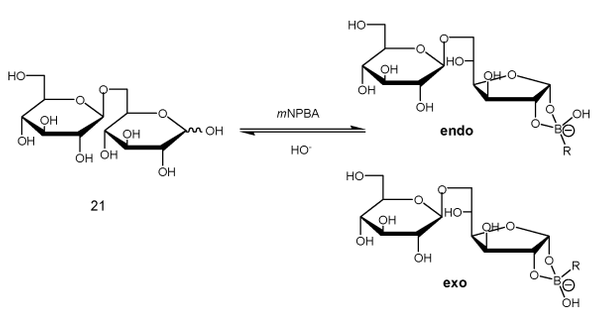

Fig. 14 1,2 *endo* and *exo* isomers of gentiobiose boronate esters indicating initial ring rearrangement from the pyranose to furanose form.

The final two compounds screened were sucrose (**23**) and palatinose (**24**). These are examples of glucose linked to fructofuranose with only the position of attachment being different. But it is in fact this difference which means that sucrose does not form a complex with boronic acids whereas palatinose does. An examination of the molecules shows that sucrose has its glucose anomeric hydroxyl linked to fructofuranose at the 2 position which is anomeric, and as all fructose boronic acid complexes involve this site interaction is understandably negated. On the other hand palatinose has the linkage at the fructofuranose 6 position and as such complexation will occur with the 2,3 coplanar diol group possibly only as the *exo* isomer due to steric inhibition from the glucose ring (*cf.* Fig. 4 structure c). Proton NMR clearly shows an unchanged spectrum for sucrose, and although proton signals and chemical shifts have not been comprehensively assigned for palatinose there is no doubt that complexation has occurred (Fig. 15).

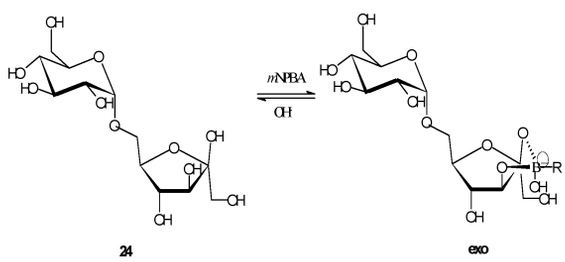

Fig. 15 Proposed interaction of palatinose with aromatic boronic acids showing complexation at the fructofuranose 2,3 position.

Interestingly the degrees of saccharide complexation with *m*NPBA presented in Tables 2 and 3 can be compared with previously published stability constants.[3,10,25,37,40] These are shown in Table 4 where the variation in association constants is tabulated against the different experimental conditions of each study. The trend and correlation of binding strengths reported by other groups matches well with the degrees of complexation found in our study. Most notably it can be seen that ketoses are more strongly bound than aldopentoses, which in turn have higher binding constants than aldohexoses. A more thorough investigation needs to be performed to fully explain these observations.

Modelling of α-galactose boronic acid complexes

Boronic acid complexes were modelled using semi-empirical QM calculations (MOPAC) using the AM1 method. The model system phenylboronic acid (PBA) was used in the modelling studies instead of *m*NPBA (as in the Experimental section) as we were not confident of the parameterisation of the nitro group in the AM1 method. Calculations with PBA are expected to give very similar results to those with *m*NPBA. Three sets of calculations were performed.

1. Calculations of different furanose and pyranose conformations of α-galactose that included different envelope and twist forms of the 5-membered ring and different chair, boat and twist boat (or twist chair or skew forms) of the 6-membered rings.

2. Calculations of one PBA complexed to the low energy diol conformations obtained from 1. For furanose sugars, 1,2,5

Table 4 Literature stability constants (log *K*) of saccharides used in this study

Saccharide	Ref. 10	Ref. 40	Ref. 37	Ref. 25	Ref. 3	Degree of complex[n]
5. Glucose	0.66	2.04	1.85	1.81	2.80	55%
6. 1-*O*-Me-Glucopyranoside					0	0%
8. 3-*O*-Me-Glucopyranoside						59%
9. Galactose	1.18	2.44		2.10		65%
10. 1-*O*-Me-Galactopyranoside						0%
12. Mannose	1.11	2.23				24%
13. Arabinose	1.40	2.59		2.10		74%
14. Xylose	1.15			2.20		68%
15. Ribose	1.38					65%
16. Fructose	2.20	3.64	3.80	3.23		Complex formed[a]
17. Sorbose	2.08			3.30		Complex formed[a]
18. Tagatose	2.11			3.30		Complex formed[a]
19. Cellobiose			1.48			6.5%
20. Maltose	0.40			1.04		5%
21. Gentiobiose			2.40			51.5%
22. Melibiose			2.76			63%
23. Sucrose	0		0	0.6		0%
24. Palatinose			3.53			Complex formed[a]

[a] Degree of complex[n] difficult to determine due to the complex nature of NMR spectra.

Table 5 Minimised structures of α-galactofuranose using AM1

Name	hof[a]	O1–O2[b]	O1–C1–C2–O2[c]	O4–C4–C5–O5	O5–C5–C6–O6	Ring conf.
fur27	−304.5	2.72	26	25	59	E (C1)
fur24	−303.7	2.62	12	77	−54	E (O4)
fur42	−303.4	2.68	25	−6	61	T
fur25	−303.4	2.64	23	3	60	T
fur45	−302.9	2.70	24	30	172	E (C2)
fur32	−302.2	2.70	14	95	−53	T
fur23	−301.5	2.63	2	81	−52	P
fur31	−301.1	2.70	20	−171	−175	E (C3)

[a] hof = heat of formation in kcal mol^{-1}. [b] In angstroms, see Fig. 16. [c] Torsion angles in degrees.

tridentate and 1,2 bidentate complexation was tried. For pyranose rings, complexes across all possible oxygen positions (1,2; 2,3 and 3,4) were tried.

3. Calculations on an additional PBA complexed to the pyranose mono esters referred to in 2 above.

α-Galactofuranose modelling

Semi-empirical QM calculations (using the AM1 method) were performed on a series of α-galactofuranose conformations (see Table 5). The different conformations generated were characterised by:

1. The conformations in the ring *i.e.* envelope (E), twist (T) and planar (P). Where the conformation is an envelope the atom not in the plane of the other four ring atoms is given in brackets.

2. The different conformations on the two rotatable bonds outside the ring.

Table 5 gives minimised conformations along with the heats of formation (hof) and conformational parameters. The table also includes the distance between O1 and O2, where PBA is expected to complex, and relevant torsion angles (see Fig. 16).

From the above list, calculations were performed on complexes with PBA for the 1,2,5 tridentate complex and both *exo* and *endo* configurations of the phenyl ring as bidentate complexes. It will be noted that the tridentate complex will form when the 5-OH has a gg orientation which corresponds to a torsional angle (O4–C4–C5–O5) of approximately +60°, and as such fur23 and 24 of Table 5 are most conducive to this formation. In these cases the oxygens are suitably orientated that the tridentate complex can easily form. All tridentate calculations result in one minimised complex. For bidentate complexes more possibilities exist. The lowest energy conformations of the bidentate complexes are given in Table 6.

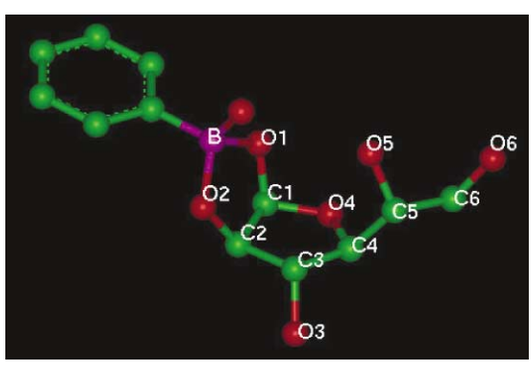

Fig. 16 α-Galactofuranose complexed with PBA. Entry 1 of Table 6.

It is evident from Table 6 that *exo* phenyl configurations are preferred in the low energy bidentate complexes (see Fig. 16). The variability in the conformations is given by the conformations outside the ring. These often differ in the hydrogen bonding between the different OH groups.

α-Galactopyranose modelling

The semi-empirical AM1 calculations on α-galactopyranose were done in 3 stages:

1. Initially different ring conformations of the pyranose ring were explored.

2. Phenylboronic acid complexes (with only one PBA) with representative conformations from the above were studied in all possible diol positions.

3. Finally complexes with 2 PBA's were studied.

Of the various chair, boat, and skew (twist boat and twist chair) conformations of the pyranose rings studied using AM1, chair conformations ($^4C_1$) were found to be lower in energy than

Table 6 Minimised structures of α-galactofuranose complexed to 1 PBA

Name	hof[a]	O4–C4–C5–O5	O5–C5–C6–O6	Ring conf.	Phenyl config.
fur25_pba	−424.0	54	50	E (C1)	*exo*
fur27_pba	−423.9	50	50	T	*exo*
fur23_pba	−422.7	103	−42	P	*exo*
fur42_pba	−422.5	8	57	E (O4)	*exo*
fur27_pba	−421.5	59	49	E (C1)	*endo*
fur45_pba	−421.1	53	146	T	*exo*
fur31_pba	−421.0	−174	160	E (C4)	*exo*
fur13_pba	−421.0	−176	−41	P	*exo*

[a] hof = heat of formation in kcal mol^{-1}.

Table 7 Minimised conformations of α-galactopyranose with 1 PBA

Name	hof[a]	Oxygens in complex	O1–O2	O3–O4	Final ring conf.	PBA config.
pyr6_pba1	−422.3	O1–O2	2.39	2.76	B	*exo*
pyr17_pba1	−421.3	O3–O4	2.85	2.38	B	*exo*
pyr5_pba1	−421.0	O1–O2	2.36	2.68	TB	*exo*
pyr5_pba2	−420.6	O1–O2	2.36	2.67	TB	*endo*
pyr6_pba0	−420.5	O1–O2	2.40	2.71	B	*endo*
pyrb0_pba0	−420.3	O1–O2	2.37	2.64	TB	*endo*
pyrb0_pba1	−420.1	O1–O2	2.37	2.66	TB	*exo*
pyr6_pba3	−419.5	O3–O4	2.64	2.38	B	*exo*

[a] hof is in kcal mol^{-1} and all distances are in angstroms. B = boat, TB = twist boat.

boat or skew ring conformations as expected. On the other hand it was found that boat and twist boats have oxygen distances between diol groups marginally smaller than the chair.

The results of one PBA complexing with α-galactopyranose rings is reported next. Starting conformations of the pyranose rings included boat, skew and chair forms. In the pyranose form 3 possible diols exist. Positions 1,2; 2,3; and 3,4. Complexes of 1 PBA in various likely positions were tried. The conformations of PBA complexed to position 2,3 were very high in energy and are not reported. Table 7 gives the different conformations of α-galactopyranose studied by AM1.

The most noticeable feature of the calculations is the conformation of the pyranose ring after the complexation. Most of the rings end up as boat or twist boat conformations. Also, the PBA in the *exo* configuration is invariably lower in energy than the *endo* configuration and, in many instances, the complexation to the oxygens at positions 1 and 2 are lower in energy than the complexes with 2,3.

It is possible that upon complexation of galactopyranose with **1** PBA, the 3,4 diol re-orientates itself such that it is conducive for complexation with another PBA. An example of a pyranose ring complexed to **1** PBA is shown in Fig. 17.

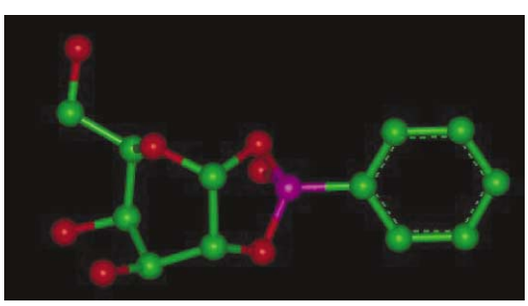

Fig. 17 α-Galactopyranose complexed with **1** PBA. Entry 1 of Table 7.

From the low energy conformations of **1** PBA complexed to the pyranose ring, complexes with another PBA molecule were studied. This gave rise to complexes with all possible combinations of PBA configurations in the complex. The lowest four energies are reported in Table 8.

Table 8 Minimised conformations of α-galacto pyranose complexed to 2 PBA's

Name	hof[a]	PBA1 config.	PBA2 config.
pyr6_pba1_pba0	−479.2	*exo*	*exo*
pyr6_pba1_pba1	−478.7	*exo*	*endo*
pyr6_pba1_pba2	−476.9	*endo*	*endo*
pyr6_pba1_pba3	−475.9	*endo*	*exo*

[a] hof is in kcal mol^{-1}.

As can be seen from Table 8 all combinations of PBA configurations have been taken into account. The lowest energy in each case is when both PBA's are in *exo* configurations. The difference between '*exo endo*' and '*endo exo*' arises from the fact that the molecule is not strictly symmetrical. The first four entries in the above table are show in the Fig. 18.

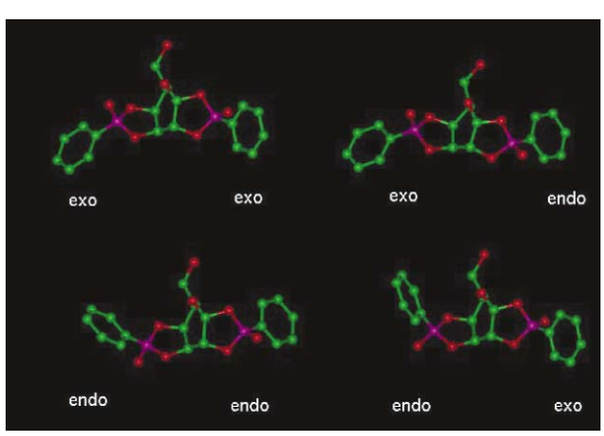

Fig. 18 The four different structures of α-galactopyranose complexed to 2 PBA's.

A systematic study of α-galactofuranose and galactopyranose rings, starting from ring conformations, and complexation to 1 and 2 phenylboronic acids suggests that:

1. For furanose conformations the lowest energy arises when 1 PBA complexes with the diol group in the 1,2 position of the ring and upon complexation the ring is nearly planar. The

variability of these complexes may arise from the rotatable bonds outside the ring and the hydrogen bonds that come into play for different conformations of these bonds. Calculations also show that certain furanose conformations are conducive to good tridentate complexation.

2. For pyranose rings, it is possible that complexation of a single PBA occurs first with the diol in position 1,2 and this reorientates the sugar ring such that another PBA can complex with the diol in position 3,4. The variability present is because of the different configurations of the two PBA groups in the final complex.

The modelling studies are in line with the experimental NMR results and confer additional support to the analysis of the ^{1}H spectra and the hypothesis of boronic acid complex formation with cyclic carbohydrates.

Conclusions

Using information taken from literature sources coupled with our own ^{1}H NMR experimental observations and molecular modelling, a hypothesis of how and under what conditions aromatic boronic acids would form stable aqueous alkaline complexes with cyclic carbohydrate substrates has been proposed. Our ideas are based on the assertion that interacting saccharides must necessarily possess a synperiplanar diol functionality for complexation to occur which is most likely with planar furanose structures. However, in circumstances where only monoboronate esters are possible, it is speculated that an energetic advantage can be achieved by diboronate ester formation with pyranose twist conformers providing that the carbohydrate has the requisite 1,2 : 3,4 polyol stereochemistry, specifically axial,equatorial : equatorial,axial or equatorial, axial : axial,equatorial. This is borne out by molecular modelling studies for the case of galactose. By observing these criteria we are enabled to predict individual carbohydrate boronic acid interactions and to propose structural assignments for complexed components. This has led to the successful screening of twenty four commercial polyhydroxy materials using ^{1}H NMR analysis to monitor complexation with aromatic boronic acids. It has been found that all of these materials produce complexation spectra in line with expectation. In summary, this study has shown that a combination of simple spectroscopic techniques coupled with inexpensive molecular modelling calculations can provide a wealth of information on the structure of complexed carbohydrates, which in turn could be a useful tool in the field of molecular sensing and recognition in aqueous media.

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