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Studies on the boronation of methyl-β-D-cellobioside—a cellulose model

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ABSTRACT

The conversion of phenylboronic acid (PBA) with methyl-β-D-cellobioside (Me-β-D-clb) and cellodextrins (DP_w 12) was investigated to gain a basic understanding of the interactions of boric acid derivatives with oligo- and polyglucans. By means of MS and NMR experiments, it was possible to show a first stage formation of a six-membered ring at C-4 and C-6 of the non-reducing glucose occurs as in the case of monosaccharides. If the amount of reagent is increased the formation of seven-membered rings at the secondary OH moieties is observed. Even the existence of two of these large ring-systems in the direct neighborhood was found. Application of an excess of boronation reagent led to dimerization reactions of Me-β-D-clb via the primary reducing glucose residue as confirmed by DOSY NMR studies. Preliminary ^{13}C NMR studies for the interaction of cellodextrins with PBA in DMSO solution confirmed a functionalization at the *trans*-1,2-diol moieties of these oligomers. The amount of reagent applied may either was shown to lead to soluble products or to insoluble cross-linked material.

1. Introduction

The benefit of boron containing compounds, for example, borax, boric acid, and boronic acids, as activating, protecting, and cross-linking agents for carbohydrates is well recognized.[1–3] Nevertheless most of the structures formed with hydroxyl moieties of saccharides are not well understood. Therefore, the exploitation of promising approaches such as the development of new derivatizing solvents for cellulose on the basis of such interactions is still limited. The boronation of monosaccharides (mainly glucose) with boronic acids has been widely applied for the development of fluorescence sensors.[4] Glucose reacts preferable in its furanose structure to build α-D-glucofuranose-1,2:3,5,6-bis(borate/boronate) complexes in alkaline aqueous media.[5] However, this rearrangement to the furanose form, which gives the required diol coplanarity for the formation of cyclic complexes, is not conceivable for glucose-based oligo- or polymers. The interaction of the most abundant polyglucans, starch, and cellulose, with boric or boronic acid-type compounds is limited to the *trans*-1,2-diol system at C-2 and C-3, and conversion of the primary hydroxyl group at C-6. Therefore this study attempted to understand the conversion of boronic acids with glucopyranose-based compounds, specifically the reaction of phenylboronic acid (PBA) with methyl-α-D-glucopyranoside (Me-α-D-Glcp).[6] Fast formation of a six-membered ring through C-4 and C-6 and the existence of a seven-membered diboronate ring at the *trans*-1,2-diol moieties of C-2 and C-3 was proven by means of NMR and MS, and 'coordination-induced shifts' (CIS) for ^{13}C NMR spectra were revealed. This new tool could now be exploited to investigate the more complex structures which may be formed in the case of the boronation of glucopyranose-based oligomers, especially cellodextrins. In contrast to the reaction of Me-α-D-Glcp, where the single primary OH-function is easily boronated under formation of a six-membered ring, boronation under cross-linking via OH-6 moieties has to be considered for oligomers. Moreover, the question arises if a dense boronation of the molecules along the chain is possible, which would result in seven-membered diboronate rings in direct neighborhood.

Recent NMR studies on D-cellobiose in alkaline aqueous sodium tetraborate solutions revealed the formation of a borate complex with the α-pyranose twist conformer at the reducing glucose end.[7] In case of non-aqueous media, Kuzuhara and Emoto suggested a conversion of cellobiose into lactose using PBA as an appropriate reagent for protection of hydroxyl groups, but there was no spectroscopic evidence of cyclic boronate structure at the cellobiose.[8] A detailed knowledge of the underlying structures is indispensable for a defined exploitation of such interactions toward activation or protection of oligo- and polyglucans. Consequently, in this paper we report on the MS and NMR spectroscopic analyses of boronation products of methyl-β-D-cellobioside (Me-β-D-clb) and cellodextrins.

2. Results and discussion

Initially, an efficient preparation of the methyl-β-glycoside of D-cellobiose starting directly from the unmodified disaccharide was established (Scheme 1). Here, the first step was the acetobromination of D-cellobiose via in situ generation of hydrogen bromide in

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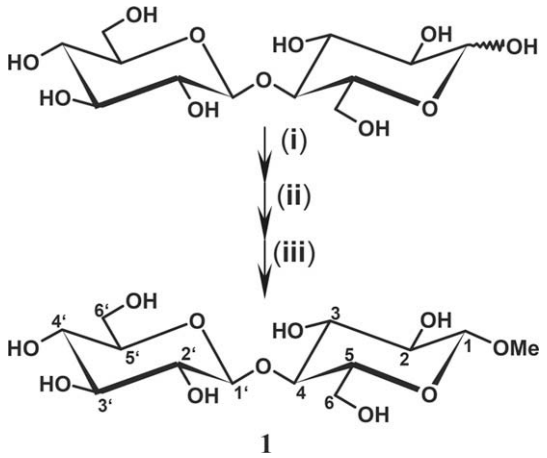


Scheme 1. Synthesis of methyl-β-D-cellobioside (Reagents: (i) Ac_2O, AcOH, H^+, AcBr, MeOH;[9a] (ii) Ag_2CO_3 in MeOH/CH_2Cl_2; (iii) NaOMe in MeOH[9b]).

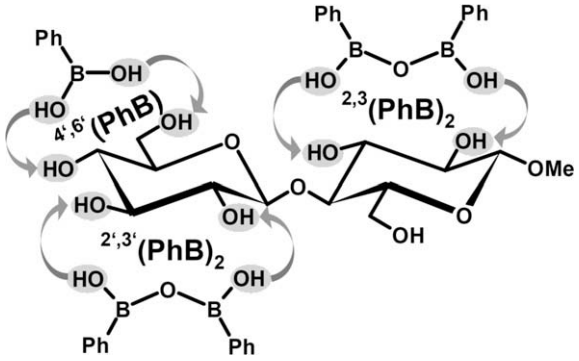
the quaternary system acetic anhydride–acetic acid–acetyl bromide–methanol, yielding the glycosyl bromide.[9a] Methylation was performed under standard Koenigs–Knorr conditions using methanol and methylene chloride in the presence of silver carbonate. The corresponding methyl-β-glycoside was deacetylated under Zemplén conditions by means of sodium methoxide in methanol.[9b] After recrystallization in ethanol the purified Me-β-D-clb **1** was obtained and characterized by NMR spectroscopy. The proton and carbon resonances were assigned using a combination of 1D-TOCSY (total correlation spectroscopy) with selective excitations, HSQC (heteronuclear single quantum correlation) and HMBC (heteronuclear multiple bond correlation) experiments and revealed the purity of the product in agreement with the literature data (see Tables S1 and S2 in Supplementary data).[10]

2.1. MS studies

For mass spectrometry (MS) studies solutions of Me-β-D-clb and triphenylboroxole (TPB) in aprotic organic solvents or water were evaporated in MS probe crucible comparable to the conversion of Me-α-D-Glcp.[6,11] After introduction into the mass spectrometer, the spectra were measured using electron impact (EI) ionization. All samples with molar ratio Me-β-D-clb:TPB higher than 1:1 showed fragment ions at *m*/*z* 250, which is a hint for the existence of a seven-membered diboronate ring at *trans*-1,2-diol moieties at C-2 and C-3. This was confirmed by MS experiments using chemical ionization (CI) with water as reagent gas. In the spectrum a peak for a molecular ion at *m*/*z* 632 was identified. This peak is assigned to a derivatized Me-β-D-clb with boronation in position 4′ and 6′ (six-membered boronate) and boronation at one *trans*-1,2-diol moiety at C-2 and C-3 or C-2′ and C-3′ (a seven-membered diboronate structure, see Table 1, entry 3). An additional indication is the isotope pattern of the peak at *m*/*z* 632 revealing the existence of three boron atoms in the detected phenylboronate structure. The isotope pattern of mass peaks is significant for boron-containing compounds because of the unique isotope distribution of boron (^{10}B:^{11}B = 1:4.2).

MS experiments using fast atom bombardment (FAB) ionization again show the peak at *m*/*z* 632 and additionally one signal at *m*/*z* 546 (Table 1, entry 2), which corresponds to one diphenylpyroboronate structure at *trans*-1,2-diol moiety. Occurrence of one seven-membered diboronate structure (*m*/*z* 546) and a six-membered boronate ring (*m*/*z* 442, cp. Table 1, entry 1) was confirmed by nano electrospray ionization mass spectroscopy (ESI-MS), but none of these techniques gave a hint for conversion of the two *trans*-1,2-diol moieties at neighboring glucose units in one step.

Table 1
Phenylboronate structures of methyl-β-D-cellobioside and the detectability with different spectroscopic methods



Entry	Structure abbreviation	Mass	Detectability
1	Me-$^{4',6'}$(PhB)-β-D-clb (**2**)	442	NMR, ESI, MALDI-TOF
2	Me-$^{2,3}(PhB)_2$-β-D-clb or Me-$^{2',3'}(PhB)_2$-β-D-clb	546	ESI, FAB, MALDI-TOF
3	Me-$^{2,3}(PhB)_2$-$^{4',6'}$(PhB)-β-D-clb or Me-$^{2',3'}(PhB)_2$-$^{4',6'}$(PhB)-β-D-clb	632	FAB, CI, MALDI-TOF
4	Me-$^{2,3}(PhB)_2$-$^{2',3'}(PhB)_2$-β-D-clb	736	MALDI-TOF
5	Me-$^{2,3}(PhB)_2$-$^{2',3'}(PhB)_2$-$^{4',6'}$(PhB)-β-D-clb (**3a**)	822	NMR

Therefore, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS was carried out. A multiple-layer spotting technique with three layers was found to be an appropriate sample preparation for saccharide boronates. It was performed as follows: first 2,5-dihydroxybenzoic acid (DHB) solution in THF was spotted on the MALDI target, as the second layer sodium iodide solution in acetone was applied, and after evaporation of organic solvents the aqueous sample solution (mixture of Me-β-D-clb and TPB with concentration between 5–10 g/L) was added on top as the third and final layer. The removal of water and consequently the esterification of hydroxyl groups with PBA units were accelerated by a drying step in a desiccator under diminished pressure.

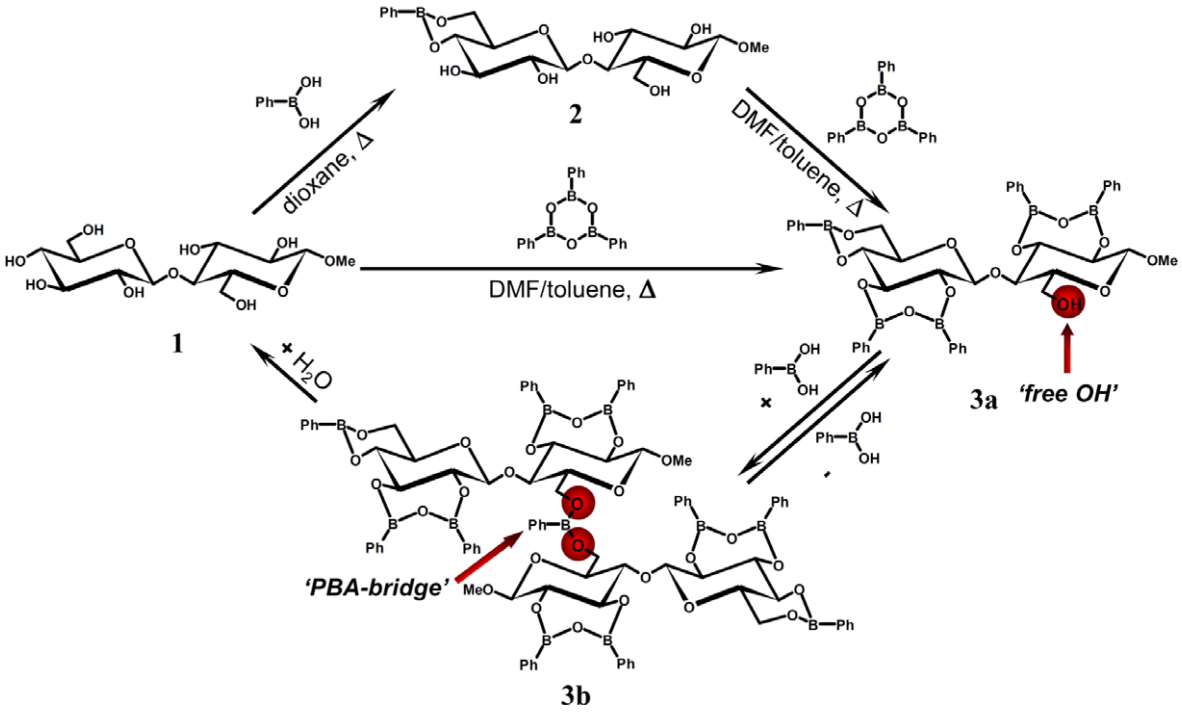
Figure 1 shows a MALDI-TOF MS spectrum (reflector mode) of an evaporated aqueous solution of Me-β-D-clb and TPB (molar ratio 1:0.6) on DHB and the peak assignment. Remarkable are the recurring mass differences ($\Delta m/z$ 86 and $\Delta m/z$ 104), which may originate from an additional PBA unit. Thus, the molecular ions $[M+Na]^+$ at *m*/*z* 465, *m*/*z* 569, *m*/*z* 655, and *m*/*z* 759 correlate with boronate structures with different numbers of boronic acid units (Table 1, entries 1–4). The detected molar masses confirm the transformation of secondary OH-groups in position 2 and 3 in addition to the expected six-membered ring at C-4′ and C-6′. Moreover, there is the molecular ion at *m*/*z* 759, which is consistent with an esterification product with two seven-membered pyroboronate rings at neighboring glucose units. So, a multi-functionalization with diboronate moieties along an oligomer- or polymer chain was found for the first time. Samples with other molar ratio of Me-β-D-clb:TPB and with α-cyano-4-hydroxy cinnamic acid (HCCA) as the matrix resulted in comparable spectra, confirming the existence of two seven-membered rings at one Me-β-D-clb molecule. Further evidence is gained from the comparison of patterns and intensities of mass peaks with calculated isotope patterns. On the basis of the isotope distribution of carbon and boron, the patterns for the main molecular ions were simulated (insets of Figs. 1 and S1 in Supplementary data). A perfect fit of the predicted pattern with the peaks for molecular ions $[M+Na]^+$ was found. Up to now no signal for an esterified product with one six- and two seven-membered rings (Table 1, entry 5) could

be detected, which would yield a peak at *m*/*z* 845. The absence or the low intensity of such a molecular ion may be due to the experimental conditions, thus, alternative techniques (matrices and spotting approaches) are under investigation.

Besides the boronate ring formation at Me-β-D-clb, MALDI-TOF MS spectra show the formation of larger adducts when higher concentrations of TPB are applied. Dimerization of two molecules Me-β-D-clb with boronic acid moieties was concluded from the peaks at *m*/*z* 821, *m*/*z* 907, and *m*/*z* 993. The molecular ions at *m*/*z* 619 and *m*/*z* 705 in the spectrum are most likely caused by the formation of Me-β-D-clb DHB adducts bridged by one or two PBA units.

2.2. Synthesis of phenylboronates and NMR analyses

In addition to Me-β-D-clb boronates formed 'in situ' during MS experiments, it was attempted to synthesize phenylboronate samples of the disaccharide based on methods established for Me-α-D-Glcp and to analyze these compounds by NMR measurments.[6,12] Thus, the esterification of Me-β-D-clb with PBA was carried out in



Scheme 2. Synthesis of phenylboronates (**2** and **3**) of methyl-β-D-cellobioside.

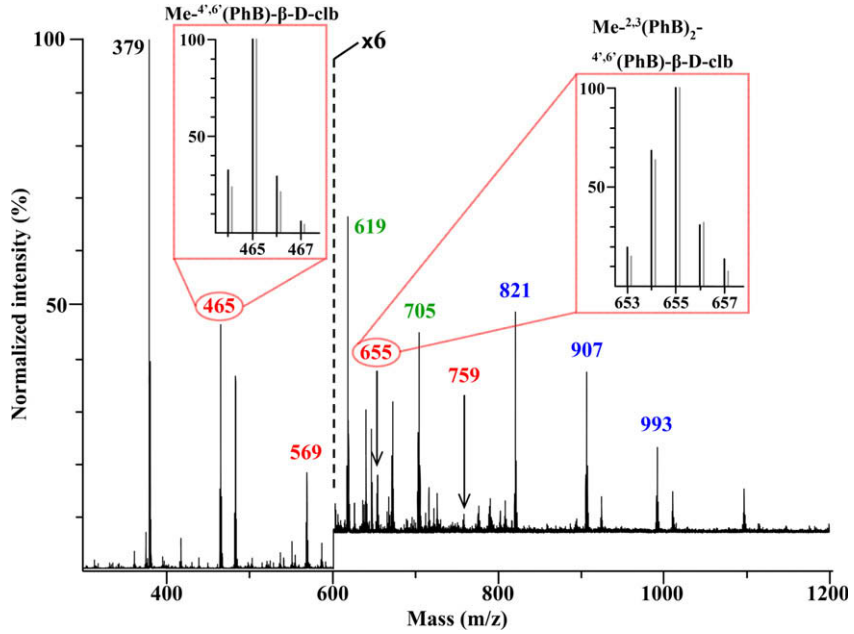


Figure 1. MALDI-TOF MS spectrum of an aqueous solution of Me-β-D-clb and TPB (molar ratio 1:0.6; matrix: DHB in THF; salt: NaI in acetone). The insets show the comparison of calculated isotope pattern (grey) with molecular ions of Me-$^{4',6'}$(PhB)-β-D-clb and Me-^{2,3}(PhB)$_2$-$^{4',6'}$(PhB)-β-D-clb.

aprotic solvents by azeotropic removal of water using a molar ratio 1:1.1. In the ^{1}H NMR spectrum of the phenylboronate sample **2** (Scheme 2) five instead of seven signals for unmodified OH moieties between 5.5 and 4.0 ppm were found. Consequently, esterification of two hydroxyl groups with PBA occurred. Figure 2 displays the 2D HMBC NMR spectrum of sample **2**. The two signals for H-6′a/b, which are shifted downfield (0.5 ppm) in comparison to signals of unmodified Me-β-D-clb, have cross-peaks with an upfield-shifted (9.1 ppm) carbon atom in position 5′. The proton of the unmodified primary hydroxyl moiety in position 6 of the methylated reducing end group (4.59 ppm) couples via two bonds with the carbon in position 6. Along the glycosidic bond there is a ^{3}J-coupling of H-1′ and C-4 of the methylated reducing glucose residue. The secondary hydroxyl protons of OH-2 and OH-2′ reveal the corresponding cross-peaks with the anomeric carbons C-1 and C-1′. OH-3′ as well as OH-3 show heteronuclear *J*-coupling with directly linked carbons in position 3 via two bonds and with adjacent carbons in position 2, respectively 4 via three bonds (see inset of Fig. 2). These results illustrate that despite the high number of signals, especially in the region 73–76 ppm, complete assignment of the spectrum is possible. The full assignment of proton and carbon shifts of **2** using the experiment discussed in combination with other 2D NMR techniques (correlation spectroscopy (COSY), HSQC-DEPT) revealed that the two missing hydroxyl protons in ^{1}H NMR spectra of esterification product of Me-β-D-clb with PBA using a molar ratio of 1:1.1 of PBA are the esterified OH-functions in position 4 and 6 (cp. Tables S1 and S2 in Supplementary data). The trend of signal movement (CIS) in the ^{13}C NMR spectrum of methyl-4′,6′-O-phenylboronate-β-D-cellobioside (**2**) resulting from the formation of a six-membered boronate ring correlates with data obtained for Me-α-D-Glcp.[6] Thus, binding-site carbons are shifted downfield (C-4′ and C-6′ 2–4 ppm), whereas for adjacent carbon atoms an upfield-shift (C-5′ 9 ppm) is observed (cp. Table S2 in Supplementary data).

In the case of esterification of Me-β-D-clb with PBA using a molar ratio 1:3.5 a product with a six-membered ring at OH-4′ and OH-6′ and an additional seven-membered pyroboronate ring at one of the *trans*-1,2-diol groups of the disaccharide (cp. Table 1, entry 3) was expected on the basis of the MS- and NMR studies discussed above. Although the ^{13}C NMR spectrum of this sample is very complex, it reveals the existence of a mixture of at least four different phenylboronates, readily noticeable by the occurrence of four signals for methoxyl carbon atoms (OMe). The number of signals in the range from 70 to 80 ppm is too high for precise assignments. Nevertheless, esterification at the *trans*-1,2-diol group can be concluded from the occurrence of several signals in the region of 77–78 ppm, which is in accordance with the discussed CIS for esterified C-2 and C-3 (downfield shift of 2–4 ppm).[6] Cross-linking of two derivatized Me-β-D-clb molecules via OH-6 of the methylated reducing glucose residue with a phenylboronic acid residue can be excluded at a molar ratio smaller than 1:3.5 because there is no second downfield-shifted carbon signal for C-6, which would support this assumption. Consequently, the complex nature of the spectra can be attributed to an equilibrium between species carrying either one or two seven-membered rings.

The phenylboronate sample **3** was obtained, if the esterification of Me-β-D-clb (molar ratio 1:5.5 of PBA) or Me-$^{4',6'}$(PhB)-β-D-clb (**2**) was carried out with an excess of reagent. From the occurrence of two singlets for methyl protons of anomeric methoxyl group (OMe) in the ^{1}H NMR spectrum (**3a**: 3.60 ppm and **3b**: 3.54 ppm), the formation of two phenylboronate structures (**3a** and **3b**) was concluded. By means of 1D NOESY NMR experiments

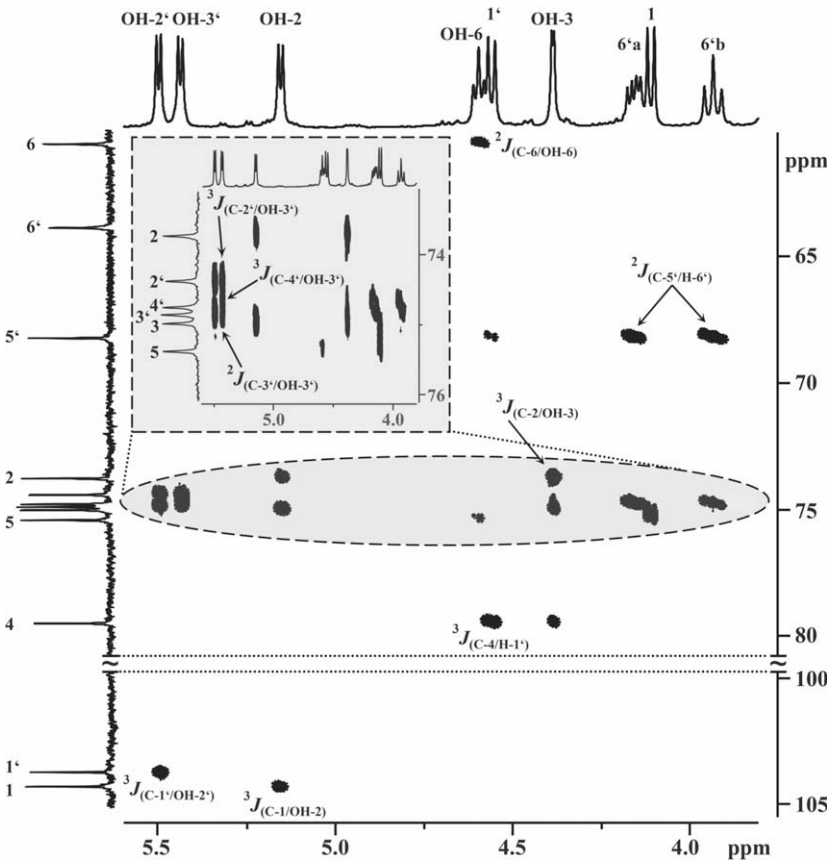


Figure 2. 2D [^{1}H, ^{13}C] HMBC NMR spectrum of Me-$^{4',6'}$(PhB)-β-D-clb (**2**) in DMSO-d_6.

(nuclear Overhauser effect spectroscopy) with selective excitation frequencies of the methoxyl protons (mentioned above) the doublets of the anomeric protons at the methylated reducing glucose residue (H-1) of both structures could be identified (see Fig. S2 in Supplementary data; δ (H-1$_{(\mathbf{3a})}$) = 4.59 ppm; δ (H-1$_{(\mathbf{3b})}$) = 4.62 ppm). The shifts for anomeric protons of the non-reducing glucose residue (H-1′) could be allocated by using the intensity ratio of the OMe signals and the H-1 proton signals (integral parts of OMe$_{(\mathbf{3a})}$ and H-1$_{(\mathbf{3a})}$ are larger; δ (H-1′$_{(\mathbf{3a})}$) = 5.07 ppm, δ (H-1′$_{(\mathbf{3b})}$) = 5.12 ppm). A series of 2D [^{1}H, ^{1}H] TOCSY NMR experiments with different mixing times (12, 40, and 90 ms) allowed identification of the resonance signals for the remaining protons of the cellobioside. The position of the shifts for H-2 and H-3, and H-2′ and H-3′, respectively, can be detected by comparing the TOCSY spectra of 12 ms and 40 ms. Using the total correlation spectroscopy NMR experiment with the longest mixing time (90 ms), the signals for H-4 and H-5 and H-4′ and H-5′ can be determined (cp. Table S1 and Figs. S3 and S4 in Supplementary data). Assignment of protons H-6 and H-6′ was carried out by HSQC-DEPT NMR spectroscopy. Here the cross-peaks with opposite sign show the position of these protons (Fig. 3). The determination of the neighboring protons at C-5 and C-5′ can be managed by the combination of 2D NMR experiments mentioned above and 2D [^{1}H, ^{1}H] ROESY NMR spectroscopy (rotating frame Overhauser effect spectroscopy) as additional proof. Thus, the only resonance signal for hydroxyl protons at 4.87 ppm can be identified as OH-6$_{(\mathbf{3a})}$ because of two cross-peaks in 2D [^{1}H, ^{1}H] TOCSY spectrum (mixing time 40 ms) with H-6$_{(\mathbf{3a})}$. In contrast, structure **3b** has no unmodified primary hydroxyl group. The esterification of this OH moiety can be supported by the two downfield shifted proton signals for H-6$_{(\mathbf{3b})}$, by 0.5–0.6 ppm in comparison to the H-6$_{(\mathbf{3a})}$ for a structure with unmodified primary hydroxyl group in position 6. Therefore, it was concluded that in both compounds all secondary hydroxyl groups are esterified, meaning that besides one six-membered ring (OH-4′ and OH-6′) two seven-membered diphenylpyroboronate rings at the *trans*-1,2-diol systems of neighboring glucose units are present. The absence of a primary hydroxyl group for structure **3b** can only be explained by a dimerization of two molecules of **3a** via a 'PBA-bridge' (Scheme 2). This was investigated by means of HSQC-DEPT NMR spectroscopy (cp. Table S2 in Supplementary data and Fig. 3). All carbon shifts for both structures are identical except the peaks for C-5 and C-6, which reveals the interaction via this moiety. Thus, an equilibrium between the dimeric structure **3b** and Me-^{2,3}(PhB)$_2$-$^{2',3'}$(PhB)$_2$-$^{4',6'}$(PhB)-β-D-clb (**3a**) exists. This assumption was confirmed by a diffusion ordered spectroscopy (DOSY) NMR experiment with the phenylboronate sample **3**, because DOSY yields signals of individual components in a mixture, separated by diffusion in different rows of a 2D data matrix. The DOSY spectrum shows, that the two compounds in the sample are slightly different in the gradient dimension F_1. The difference in molecular weight of structure **3a**/**b** is rather small showing that only dimerization occurred and cross-linking of several

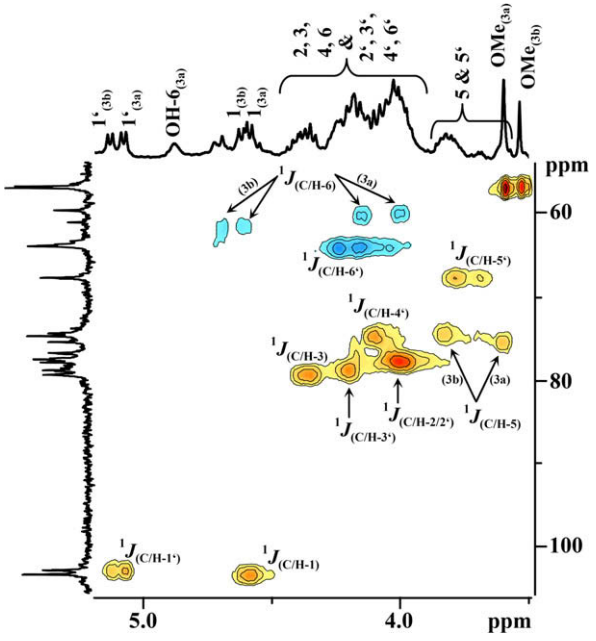


Figure 3. 2D [^{1}H, ^{13}C] HSQC-DEPT NMR spectrum of phenylboronate sample **3** consisting of Me-^{2,3}(PhB)$_2$-$^{2',3'}$(PhB)$_2$-$^{4',6'}$(PhB)-β-D-clb (**3a**), and the dimer **3b** in DMSO-d_6.

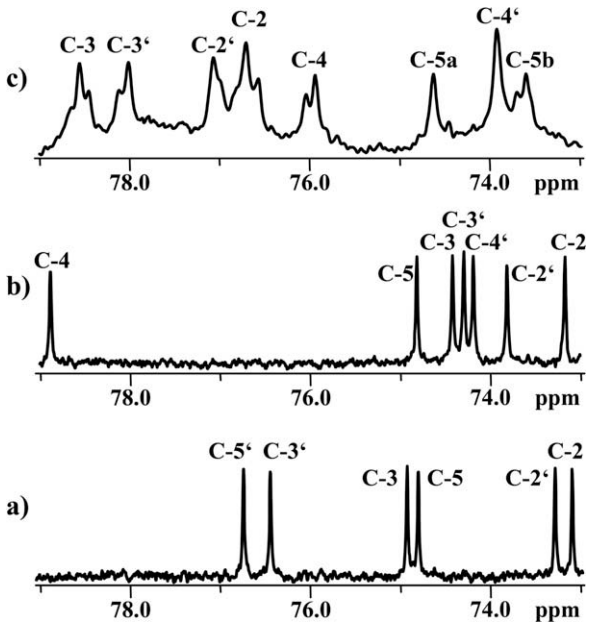


Figure 4. ^{13}C NMR spectra of (a) Me-β-D-clb (**1**); (b) Me-$^{4',6'}$(PhB)-β-D-clb (**2**); (c) phenylboronate sample **3** in DMSO-d_6.

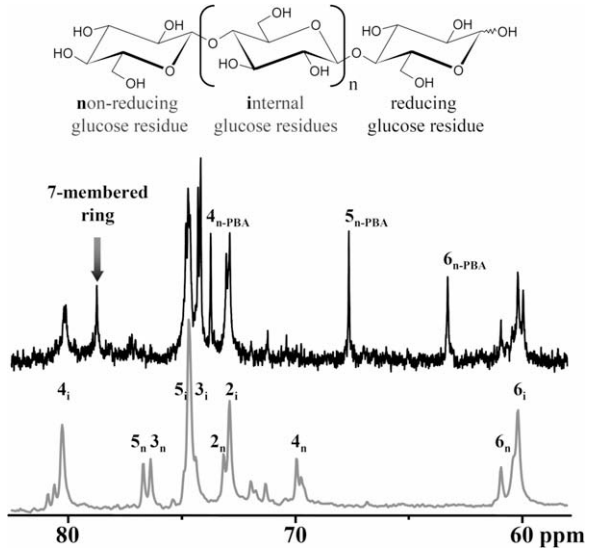


Figure 5. ^{13}C NMR spectrum of cellodextrin with DP$_w$ 12 (bottom) and cellodextrin phenylboronate sample **4** (top) in DMSO-d_6.

phenylboronates with PBA units to a polymeric structure can be excluded.

Figure 4 illustrates the trend of signal movement in ^{13}C NMR spectra as a result of esterification of Me-β-D-clb with different amounts of PBA. The assignment of all carbon signals in spectra of Me-β-D-clb (**1**) and their phenylboronates **2** and **3** allowed the comparison of CIS for binding-site carbons with the carbon atoms between or in neighborhood to the functionalized atoms. (cp. Table S2 in Supplementary data). Comparison of ^{13}C NMR spectra of Me-β-D-clb (Fig. 4a) and 4′,6′-phenylboronate **2** (Fig. 4b) shows that carbon atoms in position 2/3 and 2′, respectively, are not noticeably shifted. Pronounced signal shift was observed for the non-reducing glucose residue, especially the downfield-shift of C-4′ and C-6′ and the upfield-shift of C-3′ and C-5′. Thus, in the area between 80 and 77 ppm there are no signals apart from C-4, if no derivatization of secondary OH-groups in position 2 and 3 is present. As boronation occurs at these positions (sample **3**, Fig. 4c), signals for carbons with *trans*-1,2-diol moieties (C-2 and C-3, and C-2′ and C-3′) are shifted downfield in the formerly not occupied area. Consequently, ^{13}C NMR spectroscopy is a sensitive tool for investigation of the interaction of oligoglucans or finally cellulose with boric acid derivatives at the *trans*-1,2-diol moiety. Based on these results esterification of cellodextrins was investigated by ^{13}C NMR spectroscopy. For pure cellodextrin with DP_w 12 (weight-average degree of polymerization) no signals in the area between 80 and 77 ppm were found (Fig. 5 bottom).[13]

2.3. Preliminary studies

Boronation of cellodextrins can be carried out in the solvent system DMSO/toluene with TPB as reagent. If a molar ratio of PBA per anhydroglucose unit (AGU) smaller than 1:0.8 is applied, NMR studies are possible. At higher molar ratios precipitation from DMSO occurs, leading to badly resolved spectra.

In case of the suitable cellodextrin phenylboronate sample **4** (AGU:PBA 1:0.5) the formation of 4,6-phenylboronate at the non-reducing glucose residue is determined (see Fig. 5). Sharp signals are observed for carbons C-4 and C-6 shifted downfield with predicted values (CIS in the range of 2–4 ppm). For C-3 and C-5 an upfield-shift of 2–9 ppm is found. In the relevant area between 80 and 77 ppm a peak at 78.7 ppm appears, which indicates functionalization at the *trans*-1,2-diol system in position 2 and 3 along the cellodextrin with PBA. This is a first hint for the formation of seven-membered diboronate ring at cellodextrins.

3. Conclusion

In summary, MS analyses of phenylboronates of Me-β-D-clb and cellodextrins revealed the formation of large boronate ring structures. MALDI spectra show a multi-functionalization with diboronate moieties along the carbohydrate backbone. The comparison of isotope patterns confirms the existence of two seven-membered rings at Me-β-D-clb. A first hint for the dimerization of carbohydrate molecules with boronate moieties is observed by additional signals in MALDI spectra, especially when higher concentrations of TPB are applied. NMR analyses show that in the first step PBA binds to OH-4′ and OH-6′ of the non-reducing glucose residue forming a six-membered ring. A seven-membered pyroboronate ring at *trans*-1,2-diol group is generated if the PBA content in the reaction mixture is increased. In case of the boronation of Me-β-D-clb using a molar ratio 1:5.5 all secondary hydroxyl groups are esterified, one six- and two seven-membered rings are present. The excess of reagent leads to the dimerization of Me-β-D-clb via a 'PBA-bridge', confirmed by DOSY NMR studies. Analysis of CIS in ^{13}C NMR spectra was applied for the investigation of the interaction of cellodextrins with PBA. Besides the formation of a 4,6-phenylboronate at the non-reducing glucose residue of the oligomer, functionalization at the *trans*-1,2-diol system is indicated by a sharp signal at 78.7 ppm in ^{13}C NMR spectra.

4. Experimental

4.1. General methods

Phenylboronic acid, D-cellobiose and silver carbonate were all purchased from Sigma–Aldrich. Acetic anhydride and acetyl bromide were obtained from Acros Organics. Methanol was dried over calcium hydride. Toluene was purified by distillation. All other solvents were used as received. Triphenylboroxole was obtained by drying of phenylboronic acid in vacuum over KOH for 5 h at 200 °C, followed by sublimation of the white powder at 180 °C. The resulting mixture still contains phenylboronic acid in various amounts (^1H NMR spectrum, data not shown). ^1H and ^{13}C NMR spectra were recorded with a Bruker Avance 400 MHz instrument (400 and 100.6 MHz, respectively) at 25 °C. The two-dimensional NMR experiments (HSQC, HSQC-DEPT, HMBC, TOCSY, and COSY) were performed on Bruker Avance 400 MHz spectrometer. The concentration of all NMR samples was approximately 100 g/dm^3. MS spectra were measured with a SSQ 710 spectrometer from Finnigan MAT (EI, CI, FAB, and ESI). All MALDI experiments were performed using an Ultraflex III TOF/TOF with Nd:YAG laser and a collision cell from Bruker Daltonics, in reflector mode. MALDI samples were prepared using a multiple-layer spotting approach.

4.2. Methyl-β-D-cellobioside (1)[9a,b]

To D-cellobiose (5.0 g, 14.6 mmol) in acetic acid (50 cm^3) were added acetic anhydride (13.25 cm^3, 14.4 mmol) and perchloric acid (10 drops). The reaction mixture was stirred at room temperature for 45 min. Acetyl bromide (3.25 cm^3, 43.9 mmol) and methanol (2.0 cm^3, 59.7 mmol) were then added and the suspension was stirred at room temperature (protected from light) over night. The resulting solution was poured into ice-water (250 g) and the white solid that precipitated was filtered off. This precipitate was subjected to a normal workup (methylene chloride) to give a colorless solid. The dried solid (7.0 g, 10.0 mmol) was added to silver carbonate (3.15 g, 11.4 mmol) and 3 Å molecular sieves (3.0 g) in methanol (35 cm^3) and methylene chloride (35 cm^3). The resulting mixture was stirred for 5 h in the absence of light. After filtration through a layer of silica gel (ethyl acetate) the solution was evaporated. The obtained solid (6.2 g, 9.5 mmol) was dissolved in methanol (100 cm^3), sodium methoxide solution (30%, 2 cm^3) was added and the solution was stirred at room temperature over night. After neutralization with resin [Amberlite IR-120 (H^+)] the filtrate was concentrated. The residue was recrystallized in EtOH to yield **1** (2.7 g, 52%). ^1H NMR (400 MHz, DMSO-d_6): δ 5.17 (d, 1H, OH-2′), 5.10 (d, 1H, OH-2), 4.95 (d, 1H, OH-3′), 4.92 (d, 1H, OH-4′), 4.64 (br, 1H, OH-3), 4.54 (m, 2H, OH-6/-6′), 4.25 (d, 1H, H-1′), 4.09 (d, 1H, H-1), 3.76–3.67 (2H, H-6a/-6′a), 3.61 (m, 1H, H-6b), 3.41 (m, 1H, H-6′b), 3.38 (s, 3H, OMe), 3.32–3.22 (3H, H-3/-4/-5), 3.21–3.11 (2H, H-5′/-3′), 3.04 (m, 1H, H-4′), 3.03–2.96 (2H, H-2/-2′); ^{13}C NMR (100.6 MHz, DMSO-d_6): δ 103.5 (C-1), 103.0 (C-1′), 80.3 (C-4), 76.7 (C-5′), 76.4 (C-3′), 74.9 (C-3), 74.7 (C-5), 73.2 (C-2′), 73.0 (C-2), 70.0 (C-4′), 61.0 (C-6′), 60.4 (C-6), 56.0 (OMe).

4.3. Methyl-4′,6′-O-phenylboronate-β-D-cellobioside (2)[6]

Phenylboronic acid (197 mg, 1.6 mmol) and **1** (500 mg, 1.4 mmol) were dissolved in dioxane (100 cm^3). In a 250 cm^3

round bottom flask equipped with a Soxhlet apparatus filled with molecular sieves (3 Å) and a condenser, the solution was stirred at reflux for 5 h under argon atmosphere. After removing the solvent under reduced pressure and drying under vacuum sample **2** was obtained (590 mg, 95%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.75 (d, 2H, *o*-H_{PBA}), 7.45 (t, 1H, *p*-H_{PBA}), 7.35 (t, 2H, *m*-H_{PBA}), 5.50 (d, 1H, OH-2′), 5.43 (d, 1H, OH-3′), 5.15 (d, 1H, OH-3), 4.59 (t, 1H, OH-6), 4.56 (d, 1H, H-1′), 4.38 (d, 1H, OH-3), 4.16 (dd, 1H, H-6′a), 4.11 (d, 1H, H-1), 3.94 (t, 1H, H-6′b), 3.76 (dd, 1H, H-6a), 3.71 (3H, H-6b/-4′/-5′), 3.46–3.38 (5H, H-4/-3′ + OMe), 3.37–3.26 (2H, H-3/-5), 3.17 (m, 1H, H-2′), 3.03 (m, 1H, H-2); ^{13}C NMR (100.6 MHz, DMSO-d_6): δ 133.8 (*o*-C), 130.8 (*p*-C), 127.4 (*m*-C), 103.6 (C-1), 103.0 (C-1′), 78.9 (C-4), 74.8 (C-5), 74.4 (C-3), 74.3 (C-3′), 74.2 (C-4′), 73.8 (C-2′), 73.2 (C-2), 67.6 (C-5′), 63.3 (C-6′), 60.0 (C-6), 56.0 (OMe).

4.4. Synthesis of Me-β-D-clb phenylboronate sample (3)

1 (150 mg, 0.42 mmol) and triphenylboroxole (255 mg, 0.82 mmol) were dissolved in a mixture of *N*,*N*-dimethylformamide (15 cm^3) and toluene (35 cm^3), and it was proceeded similarly to preparation of **2** (Soxhlet apparatus filled with molecular sieve 3 Å). Removal of solvents under diminished pressure and drying under vacuum yielded sample **3** (330 mg, 95%). Compound **3a**: ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.93–7.89 (*o*-H_{PBA}), 7.42–7.33 (*m*-, *p*-H_{PBA}), 5.07 (H-1′), 4.87 (OH-6), 4.59 (H-1), 4.35 (H-3), 4.27–3.93 (H-4/-6/-2 + H-3′/-6′/-4′/-2′), 3.79 (H-5′), 3.61 (H-5), 3.60 (OMe); ^{13}C NMR (100.6 MHz, DMSO-d_6): δ 133.5–133.0 (*o*-C), 129.0 (*p*-C), 127.3–126.9 (*m*-C), 102.5 (C-1), 102.0 (C-1′), 78.6 (C-3), 78.0 (C-3′), 77.0 (C-2′), 76.7 (C-2), 76.0 (C-4), 74.6 (C-5), 73.9 (C-4′), 66.9 (C-5′), 63.4 (C-6′), 59.1 (C-6), 56.2 (OMe). Compound **3b**: ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.93–7.89 (*o*-H_{PBA}), 7.42–7.33 (*m*-, *p*-H_{PBA}), 5.12 (H-1′), 4.73–4.55 (H-6), 4.62 (H-1), 4.39 (H-3), 4.27–3.93 (H-4/-2 + H-3′/-6′/-4′/-2′), 3.84 (H-5), 3.69 (H-5′), 3.54 (OMe); ^{13}C NMR (100.6 MHz, DMSO-d_6): δ 133.5–133.0 (*o*-C), 129.0 (*p*-C), 127.3–126.9 (*m*-C), 102.5 (C-1), 102.0 (C-1′), 78.6 (C-3), 78.0 (C-3′), 77.0 (C-2′), 76.7 (C-2), 76.0 (C-4), 73.9 (C-4′), 73.6 (C-5), 66.9 (C-5′), 63.4 (C-6′), 60.6 (C-6), 56.0 (OMe).

4.5. Synthesis of cellodextrin phenylboronate sample (4)

Cellodextrin (100 mg, 0.62 mmol; $DP_n = 8$, $DP_w = 12$) was dissolved in dimethylsulfoxide (10 cm^3). After heating to 80 °C triphenylboroxole (35 mg, 0.32 mmol) in toluene (15 cm^3) was added. Under diminished pressure the toluene was removed. The step was repeated twice with additional toluene (2 × 10 cm^3). Removal of solvents under reduced pressure and drying under vacuum yielded sample **4** (125 mg, 93%). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ 135.1–127.8 (*o*-, *m*-, *p*-C), 103.0–102.7 (C-1_i), 80.2 (C-4_i) 78.7 (C-2/-$3_{i\text{-PBA}}$), 75.0–73.0 (C-2_i/-3_i/-5_i), 74.2 (C-$3_{n\text{-PBA}}$), 73.7 (C-$4_{n\text{-PBA}}$), 67.6 (C-$5_{n\text{-PBA}}$), 63.3 (C-$6_{n\text{-PBA}}$), 60.9–60.2 (C-6_i).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2009.11.007.

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