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Note

Development of a ^{1}H NMR structural-reporter-group concept for the primary structural characterisation of α-D-glucans

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Abstract—An NMR study of proton chemical shift patterns of known linear α-D-glucopyranose di- and trisaccharide structures was carried out. Chemical shift patterns for (α1→2)-, (α1→3)-, (α1→4)- and (α1→6)-linked D-glucose residues were analysed and compared to literature data. Using these data, a ^{1}H NMR structural-reporter-group concept was formulated to function as a tool in the structural analysis of α-D-glucans.
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In the structural analysis of polysaccharides, the use of NMR spectroscopy is a much employed technique. Heteropolysaccharides with a repeating unit generally have well-separated anomeric ^{1}H and ^{13}C signals for each residue of the repeating unit. The latter signals usually form the start for a complete analysis of the ^{1}H and ^{13}C NMR spectra, making use of a variety of 2D techniques, such as ^{1}H–^{1}H TOCSY, ^{1}H–^{1}H NOESY, ^{1}H–^{1}H ROESY, ^{13}C–^{1}H HSQC and ^{13}C–^{1}H HMBC. Partial acid hydrolysis fragments of the repeating unit can be easily assigned with the same NMR spectroscopic techniques. In case of homopolysaccharides such as glucans with different types of glycosidic linkages, but no repeating unit, the analysis of both the polysaccharide and the oligosaccharide fragments is more complicated. Here, to distil structural information from the various applied NMR techniques, it would be essential to know how ^{1}H and ^{13}C chemical shifts are influenced by different substitution patterns.

Besides the well-known dextran- and mutansucrase enzymes, a number of novel glucansucrase enzymes and glucan products have been characterised in recent years.[1] This includes enzymes synthesising unique α-D-glucan products, with (α1→4) or (α1→2) glycosidic bonds. The arrangement of glycosidic linkages strongly contributes to specific glucan properties such as solubility and rheology. The identification of the exact order of different glycosidic bonds in various glucan products remains a major challenge in carbohydrate research. Recently, it has been shown that the α-D-glucan produced from sucrose by glucansucrase (EC 2.4.1.5) GTFA from *Lactobacillus reuteri* strain 35-5 (**EPS35-5**) contains (α1→6) and (α1→4) linkages,[2] whereas the α-D-glucan produced from sucrose by glucansucrase GTF180 from *Lb. reuteri* strain 180 (**EPS180**) contains (α1→6) and (α1→3) linkages.[3] Mutations in the GTFA glucansucrases have led to different ratios of the linkage types.[4]

Here, we report the basics of a ^{1}H NMR structural-reporter-group concept for the analysis of such branched α-D-glucans, making use of an NMR model study of α-D-glucan di- and trisaccharides. Such concepts have been earlier developed for glycoprotein N- and O-glycans as well as for arabinoxylans.[5–7]

Samples of D-glucose, kojibiose/α-D-Glc*p*-(1→2)-D-Glc*p* (**B**→**A**; **1**), nigerose/α-D-Glc*p*-(1→3)-D-Glc*p* (**B**→**A**; **2**), maltose/α-D-Glc*p*-(1→4)-D-Glc*p* (**B**→**A**; **3**), isomaltose/α-D-Glc*p*-(1→6)-D-Glc*p* (**B**→**A**; **4**), nigerotriose/α-D-Glc*p*-(1→3)-α-D-Glc*p*-(1→3)-D-Glc*p* (**C**→**B**→**A**;

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5), maltotriose/α-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→4)-D-Glc*p* (**C**→**B**→**A**; **6**), isomaltotriose/α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→6)-D-Glc*p* (**C**→**B**→**A**; **7**) and panose/α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→4)-D-Glc*p* (**C**→**B**→**A**; **8**) were measured with ^{1}H (1D, 2D ^{1}H–^{1}H TOCSY and 2D ^{1}H–^{1}H ROESY) NMR spectroscopy, as well as 2D ^{13}C–^{1}H HSQC spectroscopy. From the data gathered, all ^{1}H and ^{13}C chemical shift values could be determined. The ^{1}H NMR data of relevance for the discussion of the ^{1}H structural-reporter-group concept are tabulated in Tables 1 and 2.

The generated NMR data, recorded at 300 K, fit earlier reported data.[8–13] It should be noted that for the development of the CASPER computer program the ^{1}H NMR data were recorded at 340 K,[14–16] yielding some differences, especially where inter-residual hydrogen bonds are involved. In principle, the CASPER computer program was developed to predict repeating-unit structures and oligosaccharides when all chemical shifts are available, in combination with substitution pattern information from methylation analysis. For homopolysaccharides, lacking a repeating unit, the CASPER computer program cannot be used for structure prediction. However, it was found to be a useful tool for verification of the assigned ^{1}H resonances.

Substitution of the reducing D-*Glc*p *unit*: Comparison of the δ values of the H-1 atoms of the reducing D-Glc*p* units **A** in oligosaccharides **1**–**8** shows that the introduction of an α-D-Glc*p* substituent at **A** O-2, O-3, O-4 or O-6 influences the exact position of the **A** H-1 resonance (Tables 1 and 2). A 2-substituted reducing Glc*p* residue [**A**; -(1→2)-D-Glc*p*; **1**] is reflected by **Aα** and **Aβ** H-1 signals at δ 5.427 and 4.785, respectively. For a 3-substituted reducing Glc*p* residue [**A**; -(1→3)-D-Glc*p*; **2** and **5**] these values are δ 5.233 and 4.663, respectively; for a 4-substituted reducing Glc*p* residue [**A**; -(1→4)-D-Glc*p*; **3**, **6** and **8**] δ 5.225 and 4.650, respectively; and for a 6-substituted reducing Glc*p* residue [**A**; -(1→6)-D-Glc*p*; **4** and **7**] δ 5.240 and 4.672, respectively. It may be clear that such differences are useful indicators of the type of substitutions in linear α-D-glucan oligosaccharide analysis.

Besides the chemical shift positions of the H-1 atoms of the reducing Glc*p* residues, also the positions of reducing β-D-Glc*p* H-2 atoms, occurring outside the bulk signal (δ 4.00–3.50) at the high-field side (δ 3.25–3.37), are similarly useful indicators of the substitution pattern of the reducing residue (Tables 1 and 2).

The importance of the position of the D-*Glc*p *H-3 signal*: The H-3 signal has a unique position for

Table 1. ^{1}H chemical shifts of D-glucopyranose residues of free D-glucose and disaccharides **1** to **4**, referenced to internal acetone (δ 2.225)

Residue[a]		D-Glucose	**1**	**2**	**3**	**4**
Aα	H-1	5.224 (3.3)	5.427 (3.4)	5.233 (3.4)	5.225 (3.7)	5.239 (3.5)
	H-2	3.524 (9.3)	3.632 (9.7)	3.63	3.56	3.55
	H-3	3.71	3.80	3.85	3.972 (9.6)	3.71
	H-4	3.402 (9.4)	3.45	3.65	3.64	3.53
	H-5	3.84	3.85	3.84	3.94	4.01
	H-6a	3.83	3.84	3.86	3.848 (1.4; −12.1)	3.70
	H-6b	3.74	3.76	3.75	3.796 (4.4)	4.00
Aβ	H-1	4.637 (7.9)	4.785 (8.3)	4.663 (7.7)	4.649 (8.0)	4.672 (7.6)
	H-2	3.236 (9.3)	3.373 (9.2)	3.332 (8.2)	3.272 (9.1)	3.253 (8.3)
	H-3	3.480 (9.4)	3.55	3.64	3.76	3.48
	H-4	3.393 (9.4)	3.43	3.64	3.62	3.52
	H-5	3.449	3.45	3.47	3.60	3.643
	H-6a	3.890 (1.9; −12.1)	3.866 (1.2; −12.5)	3.887 (1.8; −11.7)	3.902 (1.9; −12.1)	3.78
	H-6b	3.71	3.687 (4.8)	3.723 (4.8)	3.75	3.962 (4.5; −11.7)
B_α	H-1	—	5.084 (2.9)	5.371 (3.4)	5.404 (3.7)	4.948 (3.5)
	H-2	—	3.55	3.571 (9.6)	3.57	3.554 (9.8)
	H-3	—	3.78	3.75	3.68	3.75
	H-4	—	3.45	3.443 (9.6)	3.414 (9.6)	3.421 (9.4)
	H-5	—	3.932	4.014	3.71	3.75
	H-6a	—	3.84	3.84	3.848 (1.4; −12.1)	3.849 (1.4; −11.7)
	H-6b	—	3.77	3.78	3.76	3.78
B_β	H-1	—	5.376 (3.4)	5.355 (3.4)	5.404 (3.7)	4.955 (3.5)
	H-2	—	3.53	3.557 (9.6)	3.57	3.554 (9.8)
	H-3	—	3.74	3.75	3.68	3.75
	H-4	—	3.46	3.455 (9.6)	3.414 (9.6)	3.421 (9.4)
	H-5	—	4.017	4.014	3.71	3.75
	H-6a	—	3.81	3.84	3.848 (1.4; −12.1)	3.849 (1.4; −11.7)
	H-6b	—	3.78	3.78	3.76	3.78

[3] $J_{1,2}$ couplings are included between brackets, where measured.

[a] **Aα** and **Aβ** stand for the anomeric configuration of residue **A**; **B_α** and **B_β** stand for residue **B**, connected to the α- and β-anomer of residue **A**, respectively.

Table 2. ^{1}H chemical shifts of D-glucopyranose residues of trisaccharides **5**–**8**, referenced to internal acetone (δ 2.225)

Residue[a]		5	6	7	8
Aα	H-1	5.233 (3.8)	5.224 (3.4)	5.241 (3.5)	5.225 (3.9)
	H-2	3.64	3.57	3.54	3.58
	H-3	3.85	3.97	3.72	3.96
	H-4	3.66	3.63	3.50	3.63
	H-5	3.84	3.94	4.01	3.93
	H-6a	3.86	n.d.	3.70	3.86
	H-6b	3.77	3.81	4.019 (4.7; −12.3)	3.80
Aβ	H-1	4.663 (7.6)	4.650 (7.8)	4.672 (8.0)	4.650 (8.3)
	H-2	3.343 (9.3)	3.278 (9.8)	3.253 (9.2)	3.276 (9.3)
	H-3	3.65	3.77	3.48	3.77
	H-4	3.64	3.65	3.50	3.66
	H-5	3.47	3.63	3.647	3.63
	H-6a	3.89	3.91	3.78	3.91
	H-6b	3.73	3.75	3.967 (4.7; −12.3)	3.78
B	H-1	5.386/5.371 (3.8)	5.399 (3.4)	4.961/4.969 (3.5)	5.399 (3.4)
	H-2	3.68	3.63	3.57	3.60
	H-3	3.90	3.96	3.72	3.69
	H-4	3.66	3.65	3.49	3.507 (9.3)
	H-5	4.03	3.85	3.93	3.91
	H-6a	3.85	3.85	3.78	3.72
	H-6b	3.74	3.82	3.983 (4.7; −12.3)	3.97
C	H-1	5.359 (3.8)	5.399 (3.4)	4.961 (3.5)	4.955 (3.4)
	H-2	3.562 (9.7)	3.59	3.55	3.56
	H-3	3.76	3.67	3.72	3.74
	H-4	3.431 (9.2)	3.418 (9.8)	3.422 (9.6)	3.432 (9.3)
	H-5	4.01	3.72	3.72	3.73
	H-6a	3.87	3.84	3.848 (1.9; −12.3)	3.844 (2.0; −12.3)
	H-6b	3.80	3.74	3.78	3.76

[3] $J_{1,2}$ couplings are included between brackets, where measured.
[a] **Aα** and **Aβ** stand for the anomeric configuration of residue **A**.

2-, 3- and 4-substituted D-Glc*p* residues (Tables 1 and 2). In **1**, the -(1→2)-α,β-D-Glc*p* residue **A** has an H-3 signal that is shifted downfield in reference to free α,β-D-Glc*p* (α-anomer, $\delta_{H\text{-}3}$ 3.71; β-anomer, $\delta_{H\text{-}3}$ 3.48): to δ 3.80 for **Aα** ($\Delta\delta$ +0.09 ppm) and to δ 3.55 for **Aβ** ($\Delta\delta$ +0.07 ppm). In **2** and **5**, the H-3 signal of the -(1→3)-α,β-D-Glc*p* residue **A** is shifted downfield to δ 3.85 for **Aα** ($\Delta\delta$ +0.14 ppm) and to δ 3.64–3.65 for **Aβ** ($\Delta\delta$ +0.16–0.17 ppm). The chemical shift of H-3 in the -(1→3)-α-D-Glc*p*-(1→3)- unit **B** in **5** shows a similar downfield shift (δ 3.90, $\Delta\delta$ +0.15 ppm) in reference to H-3 of the α-D-Glc*p*-(1→3)- unit **B** in **2** (δ 3.75). In **3**, **6** and **8**, the -(1→4)-α-D-Glc*p* residue **A** has an H-3 signal that is shifted downfield to δ 3.96–3.97 for **Aα** ($\Delta\delta$ +0.25–0.26 ppm) and δ 3.76–3.77 for **Aβ** ($\Delta\delta$ +0.28–0.29 ppm). Here, the particularly strong downfield shift of **Aα** H-3 and **Aβ** H-3 is the result of an inter-residual hydrogen bond between **B** O-2 and **A** H(O-3).[17] The H-3 resonance of the -(1→4)-α-D-Glc*p*-(1→4)- unit **B** in **6** is shifted downfield, in reference to H-3 of α-D-Glc*p*-(1→4)- unit **B** in **3** (δ 3.68), to δ 3.96 ($\Delta\delta$ +0.28 ppm).

The importance of the position of the α-D-Glcp H-4 signal: In free α-D-glucopyranose, the H-4 signal resonates at δ 3.40, which is situated outside the bulk region. In non-reducing terminal α-D-Glc*p* units [α-D-Glc*p*-(1→*x*)-], this ^{1}H signal shows, depending on the glycosidic linkage, only a slight upfield shift (for **1**-**8**, δ 3.41–3.45). In substituted α-D-Glc*p* units, the H-4 signals may show specific downfield shifts into the bulk region (Tables 1 and 2). In case of a -(1→2)-α-D-Glc*p* unit, the H-4 resonates at δ 3.45 (**1**), which overlaps with the region of an α-D-Glc*p*-(1→*x*)- unit. However, the H-4 signal of -(1→6)-α-D-Glc*p*(-) resonates at δ 3.49–3.53 (**4**, **7**, **8**) and that of -(1→3)-α-D-Glc*p*(-) and -(1→4)-α-D-Glc*p*(-) at δ 3.63–3.66 (**2**, **3**, **5**, **6**, **8**). This means that as long as there are no (α1→2) linkages present in an α-D-glucan oligo- or polysaccharide, a peak in the range of δ 3.41–3.45 corresponds with an α-D-Glc*p*-(1→*x*) unit. In oligosaccharides, derived from a partial acid hydrolysis of polysaccharides, the surface area under this peak can be used to determine whether the fragment is a branched oligosaccharide or not. In polysaccharides, this peak may be a useful indicator of the degree of branching.

The importance of the position of the D-Glcp H-5 signal: Analysis of oligosaccharides **1**–**8** showed interesting features when focusing on the H-5 signals (Tables 1 and 2). Taking free α-D-glucopyranose as a starting point ($\delta_{H\text{-}5}$ 3.84), the H-5 signal of the -(1→2)-α-D-Glc*p* unit

Aα in **1** is found at a similar position (δ 3.85), but the H-5 signals of the α-D-Glc*p*-(1→2)- units $\mathbf{B}_{\alpha}$ and $\mathbf{B}_{\beta}$ are shifted downfield to δ 3.93 ($\Delta\delta$ +0.09 ppm) and 4.02 ($\Delta\delta$ +0.18 ppm), respectively. Taking free β-D-glucopyranose as a starting point ($\delta_{H\text{-}5}$ 3.45), the H-5 signal of the -(1→2)-β-D-Glc*p* unit **Aβ** in **1** is found at a similar position (δ 3.45). The relatively high downfield position of the **B** H-5 signals is caused by an inter-residual hydrogen bond between **B** O-5 and **A** H(O-3) in both the anomeric forms.[17]

Also in **2**, the H-5 signals of the reducing -(1→3)-α,β-D-Glc*p* unit **A** are found close to the positions of free α,β-D-Glc*p* H-5. However, the H-5 of the α-D-Glc*p*-(1→3)- unit **B** is observed at δ 4.01 ($\Delta\delta$ +0.17 ppm). The relatively high downfield position of **B** H-5 is due to the inter-ring hydrogen bond between **B** O-5 and **A** H(O-2).[17] Such a downfield shift is also found in **5**, showing α-D-Glc*p*-(1→3)- unit **C** and -(1→3)-α-D-Glc*p*-(1→3)- unit **B** H-5 at δ 4.01 [**C** O-5···**B** H(O-2)] and 4.03 [**B** O-5···**A** H(O-2)], respectively, whereas the H-5 signals of the -(1→3)-α,β-D-Glc*p* **A** unit are similar to those of free α,β-D-Glc*p* H-5.

In contrast to the H-5 positions of the reducing units in **1** and **2**, those in **3** and **6** show downfield shifts with respect to free α,β-D-Glc*p*, that is, -(1→4)-α-D-Glc*p* unit **Aα** H-5 appears at δ 3.94 ($\Delta\delta$ +0.10 ppm) and -(1→4)-β-D-Glc*p* unit **Aβ** H-5 at δ 3.60–3.63 ($\Delta\delta$ +0.15–0.18 ppm). The H-5 signals of the α-D-Glc*p*-(1→4)- unit **B** in **3** and **C** in **6** are detected at the upfield positions δ 3.71–3.72 ($\Delta\delta$ −0.13 to −0.12 ppm); the H-5 resonance of the -(1→4)-α-D-Glc*p*-(1→4)- unit **B** in **6** shifts significantly downfield (δ 3.85, $\Delta\delta$ +0.14 ppm) in reference to the H-5 of α-D-Glc*p*-(1→4)- unit **B** in **3** (δ 3.71).

In a similar way as shown for **3** and **6**, the H-5 signals of the -(1→6)-α-D-Glc*p* unit **Aα** and the -(1→6)-β-D-Glc*p* unit **Aβ** in **4** and **7** show downfield shifts: **Aα** H-5 at δ 4.01 ($\Delta\delta$ +0.17 ppm) and **Aβ** H-5 at δ 3.64–3.65 ($\Delta\delta$ +0.19–0.20 ppm). The H-5 signal of the α-D-Glc*p*-(1→6)- unit **B** in **4** and **C** in **7** are detected at the upfield positions δ 3.75–3.72 ($\Delta\delta$ −0.09 to −0.12 ppm); the H-5 resonance of the -(1→6)-α-D-Glc*p*-(1→6)- unit **B** in **7** shifts significantly downfield (δ 3.93, $\Delta\delta$ +0.18 ppm) in reference to H-5 of α-D-Glc*p*-(1→6)- unit **B** in **4** (δ 3.75).

The deduced information with respect to the H-5 fits completely with the situation in **8** (panose). Here, **Aα** and **Aβ** H-5 have chemical shifts of δ 3.93 and 3.63, respectively, in accordance with a reducing -(1→4)-α-D-Glc*p* **A** unit (compare with **4** and **6**). The **B** H-5 signal appears at δ 3.91, reflecting a -(1→6)-α-D-Glc*p*-(1→4/6)- unit (in this case -(1→6)-α-D-Glc*p*-(1→4)- unit **B**) and the **C** H-5 resonance is observed at δ 3.73, reflecting an α-D-Glc*p*-(1→4/6)- unit (in this case α-D-Glc*p*-(1→6)- unit **C**).

The importance of the positions of the α-D-Glcp H-6a,b signals: Focusing on the α-D-glucopyranose H-6a and H-6b chemical shifts (Tables 1 and 2), both the values show significant changes upon O-6 substitution of the specific residue, when compared with the corresponding free α-D-Glc*p* H-6a (δ 3.83) and H-6b (δ 3.74) values. Characteristic upfield shifts are observed for H-6a (δ 3.78–3.70) and downfield shifts for H-6b (δ 3.97–4.02) for -(1→6)-α-D-Glc*p* residue **Aα** in **4** and **7** and for -(1→6)-α-D-Glc*p*-(1→6/4)- residue **B** in **7** and **8**. A similar trend is seen for the H-6a and H-6b signals of reducing β-D-Glc*p* units.

Comparison of the data in Tables 1 and 2 indicates that the positions of the α-D-Glc*p* H-6a and H-6b signals are not significantly influenced by O-2 or O-3 substitution of the specific residue (compare free α-D-Glc*p* with **1**, **2** and **5**). However, 4-O substitution has a clear influence on the position of H-6b, revealing a downfield shift to 3.82–3.80 ppm (compare α-D-Glc*p* with -(1→4)-α-D-Glc*p* residue **Aα** in **3**, **6** and **8**; and with -(1→4)-α-D-Glc*p*-(1→4)- residue **B** in **6**).

Although the δ values of α-D-Glc*p* H-6a and H-6b can be used as clear indicators for 4- and 6-substitutions, it should be noted that in a series of 2D TOCSY experiments with increasing mixing times (10, 30, 60, 120 and 180 ms) the H-6a and H-6b signals are the last to appear in the anomeric tracks of oligo/polysaccharides. Therefore, in cases where low solubility or minute amounts of oligo/polysaccharide make the generation of a full set of TOCSY data difficult, the H-6a and H-6b cross-peaks are quite often missing.

The linearity of ^{1}H chemical shifts: Comparison of the H-1–H-6a,b chemical shift patterns (i.e., TOCSY patterns) of the non-reducing terminal α-D-Glc*p*-(1→*x*)- residues in the analysed di- and trisaccharides reveals overlapping features for the same *x*. The chemical shift pattern of the α-D-Glc*p*-(1→3)- residue **C** in trisaccharide **5** corresponds with that of the α-D-Glc*p*-(1→3)- residue **B** in disaccharide **2**. The chemical shift pattern of the α-D-Glc*p*-(1→4)- residue **C** in trisaccharide **6** corresponds with that of the α-D-Glc*p*-(1→4)- residue **B** in disaccharide **3**. The chemical shift pattern of the α-D-Glc*p*-(1→6)- residue **C** in trisaccharides **7** and **8** corresponds with that of the α-D-Glc*p*-(1→6)- residue **B** in disaccharide **4**.

Compared to free α-D-Glc*p*, in disaccharide **2** (nigerose), the **Aα** H-2, H-3 and H-4 signals are shifted downfield to δ 3.63 ($\delta_{H\text{-}2}$ 3.52; $\Delta\delta$ +0.11 ppm), 3.85 ($\delta_{H\text{-}3}$ 3.71; $\Delta\delta$ +0.14 ppm) and 3.65 ($\delta_{H\text{-}4}$ 3.40; $\Delta\delta$ +0.25 ppm), respectively. In trisaccharide **5** (nigerotriose) the **B** H-2, H-3 and H-4 resonances are shifted downfield, in reference to α-D-Glc*p*-(1→3)- unit **B** in **2**, to δ 3.68 ($\delta_{H\text{-}2}$ 3.57; $\Delta\delta$ +0.11 ppm), 3.90 ($\delta_{H\text{-}3}$ 3.75; $\Delta\delta$ +0.15 ppm) and 3.66 ($\delta_{H\text{-}4}$ 3.44; $\Delta\delta$ +0.22 ppm), respectively.

Compared to free α-D-Glc*p*, in disaccharide **3** (maltose), the **Aα** H-3, H-4 and H-5 signals are shifted downfield to δ 3.97 ($\delta_{H\text{-}3}$ 3.71; $\Delta\delta$ +0.26 ppm), 3.64 ($\delta_{H\text{-}4}$ 3.40; $\Delta\delta$ +0.24 ppm) and 3.94 ($\delta_{H\text{-}5}$ 3.84; $\Delta\delta$ +0.10 ppm),

respectively. In trisaccharide **6** (maltotriose), the **B** H-3, H-4 and H-5 resonances are shifted downfield, in reference to α-D-Glc*p*-(1→4)- unit **B** in **3**, to δ 3.96 ($\delta_{H\text{-}3}$ 3.68; $\Delta\delta$ +0.28 ppm), 3.65 ($\delta_{H\text{-}4}$ 3.41; $\Delta\delta$ +0.24 ppm) and 3.85 ($\delta_{H\text{-}5}$ 3.71; $\Delta\delta$ +0.14 ppm), respectively.

Compared to free α-D-Glc*p*, in disaccharide **4** (isomaltose), the **Aα** H-4, H-5, H-6a and H-6b signals are shifted to δ 3.53 ($\delta_{H\text{-}4}$ 3.40; $\Delta\delta$ +0.13 ppm), 4.01 ($\delta_{H\text{-}5}$ 3.84; $\Delta\delta$ +0.17 ppm), 3.70 ($\delta_{H\text{-}6a}$ 3.83; $\Delta\delta$ −0.13 ppm) and 4.00 ($\delta_{H\text{-}6b}$ 3.74; $\Delta\delta$ +0.26 ppm), respectively. In trisaccharide **7** (isomaltotriose) the **B** H-4, H-5, H-6a and H-6b resonances are shifted, in reference to α-D-Glc*p*-(1→6)- unit **B** in **4**, to δ 3.49 ($\delta_{H\text{-}4}$ 3.42; $\Delta\delta$ +0.07 ppm), 3.93 ($\delta_{H\text{-}5}$ 3.75; $\Delta\delta$ +0.18 ppm), 3.78 ($\delta_{H\text{-}6a}$ 3.85; $\Delta\delta$ −0.07 ppm) and 3.98 ($\delta_{H\text{-}6b}$ 3.78; $\Delta\delta$ +0.20 ppm), respectively. In trisaccharide **8** (panose) the **B** H-4, H-5, H-6a and H-6b signals are shifted, in reference to α-D-Glc*p*-(1→4)- unit **B** in **3**, to δ 3.51 ($\delta_{H\text{-}4}$ 3.41; $\Delta\delta$ +0.10 ppm), 3.91 ($\delta_{H\text{-}5}$ 3.71; $\Delta\delta$ +0.20 ppm), 3.72 ($\delta_{H\text{-}6a}$ 3.85; $\Delta\delta$ −0.13 ppm) and 3.97 ($\delta_{H\text{-}6b}$ 3.76; $\Delta\delta$ +0.21 ppm), respectively.

Comparison of the chemical shifts of residue **B** in trisaccharides **5**–**8** with those in disaccharides **1**–**4** shows that the size and direction (up- or downfield) of the shifts upon substitution are similar to the size and direction of the shifts of residue **Aα** of **1**–**8** in reference to free α-D-Glc*p*.

For the analysis of α-D-glucan oligo- and polysaccharides, in this study a series of structural-reporters have been defined that can be used in 1D ^{1}H NMR spectroscopy. The substitution of the reducing residue [-(1→*x*)-α-D-Glc*p*] can be derived directly from the anomeric ^{1}H chemical shifts. The amount of branching can be derived from the surface area under the peak around δ 3.40, provided there are no (α1→2) linkages. Although most of the chemical shifts fall inside the bulk region of δ 4.00–3.50, the 2D TOCSY H-1–H-6a,b patterns of each residue can easily be used for the identification of substitution patterns. Even when using 2D TOCSY experiments with short mixing times (7–60 ms), the specific resonances of H-2, H-3 and H-4 will often suffice to elucidate an oligosaccharide structure. This means that when only minute amounts of oligosaccharide or polysaccharide sample are available, or low solubility impedes 2D NMR spectroscopy, structures can still be solved.

Even though ^{13}C–^{1}H HSQC spectra do not seem to be strictly necessary for the identification of α-D-glucans, the data from this type of experiments are useful for the verification of assignments made by 2D TOCSY. The ^{13}C chemical shift is more predictable than the ^{1}H chemical shift, but it can only be used when sufficient amounts of material are available, or with ^{13}C-enriched samples.

The developed ^{1}H structural-reporter-group concept has been used successfully in the primary structural analysis of α-D-glucans secreted by *Lb. reuteri* strains, as will be reported elsewhere.

1. Experimental

1.1. Materials

Nigerose, maltose, maltotriose, isomaltose, isomaltotriose and panose were purchased from Sigma (⩾95%, HPAEC-PAD). Nigerotriose was purchased from Dextra Laboratories Ltd, Reading, UK. Kojibiose was a gift of Dr. B. H. Koeppen (South Africa). D_2O (99.9 atom%) was acquired from Cambridge isotope laboratories, Inc., Andover, MA.

1.2. NMR spectroscopy

^{1}H NMR spectra, including ^{1}H–^{1}H and ^{13}C–^{1}H correlation spectra were recorded at a probe temperature of 300 K on a Bruker DRX500 spectrometer (Bijvoet Center, Department of NMR spectroscopy). Samples were exchanged once with 99.9 atom% D_2O, lyophilised and dissolved in 650 μl D_2O. ^{1}H chemical shifts are expressed in ppm by reference to internal acetone (δ 2.225). One-dimensional 500-MHz ^{1}H NMR spectra were recorded with a spectral width of 5000 Hz in 16k complex data sets and zero filled to 32k. A WEFT pulse sequence was applied to suppress the HOD signal.[18] When necessary, a fifth order polynomial baseline correction was applied. Two-dimensional TOCSY spectra were recorded using MLEV17 mixing sequences with spin-lock times of 10, 30, 60, 120 and 180 ms.[19] The spin-lock field strength corresponded to a 90° pulse width of about 28 μs at 13 dB. The spectral width in the 2D TOCSY experiments was 4006 Hz at 500 MHz in each dimension. 400–1024 spectra of 2k data points with 8–32 scans per t_1 increment were recorded. 2D rotating-frame nuclear Overhauser enhancement spectra (ROESY) were recorded with 300 ms mixing time.[20] The spectral width was 4006 Hz at 500 MHz in each dimension. Suppression of the HOD signal was performed by a 1 s pre-saturation during the relaxation delay. Between 400 and 1024 data sets of 2k data points were recorded with 8–16 scans per t_1 increment. 2D ^{13}C–^{1}H HSQC spectroscopy was carried out at a ^{1}H frequency of 500.0821 MHz and a ^{13}C frequency of 125.7552 MHz.[21] Spectra were recorded with a spectral width of 4006 Hz for t_2 and 10,000 Hz for t_1. The HOD signal was pre-saturated for 1 s, and ^{12}C-bound protons were suppressed using a TANGO pulse sequence. During acquisition of the ^{1}H FID, a ^{13}C decoupling pulse was applied. 128–256 experiments of 2k data points were recorded with 128 scans per t_1 increment. 2D NMR spectroscopic data were analysed by applying a sinus multiplication window and zero filling to spectra of 4k

by 1k dimensions. In case of ^{13}C–^{1}H HSQC data, the spectra were zero filled to 4k by 512 data points. A Fourier transform was applied, and where necessary, a 5–15th order polynomial baseline function was applied. All NMR data were processed using in-house developed software (J. A. van Kuik, Bijvoet Center, Department of Bio-Organic Chemistry, Utrecht University).

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