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RELATIVE REACTIVITIES IN THE *O*-METHYLATION OF GLUCOMANNANS: THE INFLUENCE OF STEREOCHEMISTRY AT C-2 AND THE SOLVENT EFFECT

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Abstract

The main hemicellulose in softwood, glucomannan (GM), structurally resembles cellulose but has quite different physical and chemical properties. In addition to branching and original acetylation, the only other difference between these two β-1,4-linked glycans is the configuration at C-2 in approximately 80% of the sugar residues. In contrast to glucose, the 2-OH in mannose has an axial orientation. The influence of this stereochemistry on the relative reactivities of glucosyl compared to mannosyl units in methylation reactions are studied in this work.

Glucomannan isolated from spruce (SGM) and commercially available konjac glucomannan (KGM) were methylated in DMSO/Li-dimsyl/MeI and water/NaOH/MeI system, respectively. In the early stage of the reaction, the glucose part of the SGM achieved slightly higher DS values than the mannose residues, but the overall relative rate constants were close to 1:1. The order of reactivities in glucose was $k_2 > k_3 > k_6$ and $k_3 > k_2 > k_6$ for mannose (in DMSO/Li-dimsyl/MeI). The rate constants did not remain constant, but k_3 decreased when k_2 increased for both epimeric sugars. In water/NaOH/MeI, the methylation of the primary 6-OH was much more pronounced with an order of reactivity of O-6 > O-2 > O-3 for mannose and O-2 > O-6 > O-3 for glucose. The results are discussed with respect to the OH-acidity and the stereoelectronic, sterical and solvent effects.

Keywords

Spruce glucomannan, Relative reactivities, Partial methylation, Solvent effect

INTRODUCTION

Norway spruce (*Picea abies*) grows abundantly in Scandinavian countries and is composed of long fibers that are primarily made up of cellulose, hemicelluloses and lignin. As a typical softwood species, it contains a substantial amount of hemicellulose, which is mainly composed of mannans. Spruce mannans consist of β-1,4-D-Man*p* and β-1,4-D-Glc*p* residues with D-Gal*p* and acetyl side-groups. There are two types of mannans according to different monosaccharide ratios: one is galactose-rich with a galactose to glucose to mannose ratio of 1:1:3, termed galactoglucomannan (GGM), and the other is galactose-poor with a ratio of 0.1:1:3, termed spruce glucomannan (SGM)[1]. Compared to Konjac glucomannan (KGM), from the herb *Amorphophallus konjac*, SGM is of lower molecular weight and has a higher mannose: glucose ratio ca. 3.8: 1 (KGM ca. 1.6: 1). Both SGM and KGM show a certain degree of branching at O6 of the mannose-glucose backbone. Recently, KGM was shown to be branched at the glucosyl units and bear mannose-rich oligomeric side chains[2]; however, different opinions exist in the literature.

Esterification and etherification of hydroxyl groups are common methods to modify sugar molecules to equip natural carbohydrates with new functions and characteristics. Glucomannans and their derivatives, blends and composites have been used in the pharmaceutical, food, cosmetics and painting industries as solubilizers, adhesives, or drug delivery substrates.[3, 4] By substitution of the hydroxyl groups, the properties of the native polysaccharide can be varied over a wide range. Xu et al. [5] studied carboxymethylation of a galactoglucomannan in aqueous NaOH/isopropanol. Based on the GC-MS of monosaccharide derivatives and the NMR spectra, they conclude that the accessibility of O-6 is the highest while O-2 and O-3 are shielded by carboxymethyl substituents at O-6. Another study from the same group was aimed at enhancing the properties of spruce galactoglucomannan by acetylation. When applying different reaction conditions, the migration of acetyl groups from O-2 to O-3 to O-6 occurs, whether this happens directly or stepwise and within or between different units is not known [6]. An et al. reported the methylation of KGM to obtain water-soluble derivatives using aqueous NaOH/MeI at pH 8-10 and studied methyl KGM using ^{1}H, ^{13}C and 2D NMR spectroscopy in D_2O at 80 °C [7]. Degree of substitution (DS) values of 0.09 - 0.18 were estimated, but the positions of the methyl groups on glucose and mannose could not be differentiated. Because the assignment of OCH_3 is contradictory, the order of reactivity cannot be deduced from the signal. Vopel et al. developed cross-linked hydrogels based on acetylated galactoglucomannan. The DS was tuned by varying the steric requirements of the imidazole-activated alkenyl reagents [8].

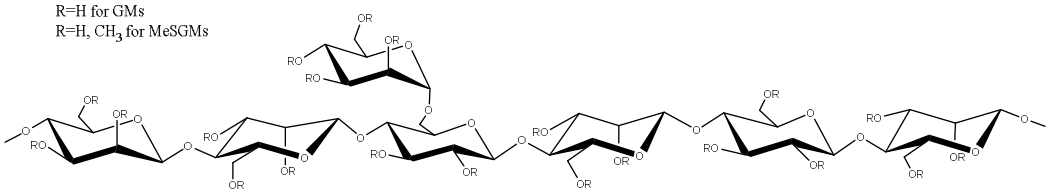

Fig. 1 Proposed structures for spruce glucomannan and its partially methylated derivatives. Approximately 20% of the units have the glucosyl-configuration, and 80% have the mannosyl configuration.

Fig. 1 shows the proposed chemical structure of SGM. Depending on the source, there are variations in the degree of acetylation and branching [9-11]. As reported by Albrecht et al. [2], little is known about the precise structure of glucomannans, especially the sequence of glucose and mannose within the backbone. In the work from Katsuraya et al.[12], the main chain and the branched structure of KGM were investigated using ^{13}C NMR. They concluded that KGM was composed of β-1,4-linked D-glucosyl and D-mannosyl residues comprising the backbone with branches through β-1,6-glucosyl units at approximately 8%, but the length of the branches is not known and may be longer than one sugar unit.

The backbone could be considered a stereoisomer of cellulose with a partial inversion at C-2. Due to the different orientation of OH at C-2, which is axial in mannose and equatorial in glucose, the relative reactivities of the free hydroxyl groups at the 2, 3, and 6 positions of glucose and mannose are expected to be different. A tunable regioselectivity during the mono-etherification of the 2,3 diol of a mannose derivative has been recently published[13].

In addition, the overall reactivities of glucosyl compared to mannosyl units are of interest. The regioselectivities will not only influence the substituent distribution in the monomer units but also the substituent pattern over the polymer chain and, thus, the physical properties [14]. Williamson-type etherification using methyl iodide (MeI) was chosen as the model reaction to study the relative reactivities of OH in mannosyl and glucosyl residues of GM because the steric requirements of the reagent are low, etherification is irreversible, and quantitative analysis on various structural levels is well established for the corresponding cellulose ethers [15, 16].

For a better understanding of the mechanisms and directed synthesis, it is essential to study the reactivity of the hydroxyl groups on different positions of the sugar units under various reaction conditions. The relative reactivities of the free hydroxyl groups in cellulose, dextran, amylose and cyclodextrin derivatives have been determined by studying their etherification patterns [16-18]. In contrast to polysaccharides composed of a single monosaccharide, the study of the reactivity of glucomannans is more complicated. Therefore, the relative reactivities of glucosyl compared to mannosyl units in etherification reactions need to be discussed for further application of this material. To the best of our knowledge, no work

regarding the individual reactivities of glucose and mannose in glucomannan has been reported so far.

A highly purified SGM [1] isolated from Norway spruce has been used in this study, and a method for evaluating the methylated glucomannans has been established. With SGM, the reactions were performed in homogeneous systems with DMSO as an aprotic polar solvent, the corresponding anion Li-dimsyl as the base and MeI as the methylating agent [19, 20]. KGM was reacted in water with NaOH and MeI for comparison.

EXPERIMENTAL

General

SGM extracted from spruce wood chips was used [1]. The acetyl groups originally present had been cleaved off due to the strong alkaline extraction condition during isolation and purification. The sugar composition of the pure material was determined to be arabinose 0.3%, galactose 2.3%, glucose 20.2%, xylose 1.4% and mannose 75.8% (all w/w). (M:G = 3.75). Commercially available mannan (Ivory nut, Megazyme Co. Wicklow, Ireland.) and methylcellulose (Sigma Aldrich) were used as standards for assigning partially methylated alditol acetates of mannose and glucose. KGM with a much higher molecular mass than SGM with M:G = 1.67 was purchased from Hubei Konson Konjac Gum Co., Ltd., China (food additive grade).

Trace elemental analysis was performed using inductively coupled plasma atomic emission spectroscopy (Thermo Scientific iCAP 6000 series ICP spectrometer, ICP-AES). The analyses was performed at wavelengths of 213.5 and 219.9 nm for copper and at 182.5 and 182.6 nm for bromium, using an ICP multi-element standard IV from Merck. The ICP elemental analysis of SGM showed 2.549 ppm B corresponding to 0.0003 mol/mol(AGU) and 13.2 ppm Cu corresponding to 0.0070 mol/mol(AGU).

All reagents were of high purity and purchased from Fluka, Aldrich or Merck. Analytical reactions were carried out in 1 mL or 5 mL V-vials in a heating block and an evaporating unit from Barkey, GmbH & Co. KG, Germany. For quantitative GLC evaluation using a flame ionization detector (FID), the effective carbon response (ECR) concept was applied [21].

Instrumental

Gas chromatographic analysis was carried out using a GLC-FID instrument Shimadzu GC 2010, equipped with a Phenomenex Zebron ZB-5HT Iferno column (30 m) and a retention gap (methyl deactivated., 1.5 m), carrier gas H_2 (65.4 kPa), split injection port 250 °C, split ratio 0, carrier gas flow 40 cm/s (linear velocity mode). Temperature program: 60 °C (1 min), with 20 °C/min to 150 °C, with 1 °C/min to 180 °C and with 20 °C/min to 310 °C (10 min). Data

were recorded with Shimadzu GC solution Chromatography Data SYSTEM Version 2.3. GLC-MS was performed on a GC 5890A (Hewlett Packard) using electron impact ionization (EI) (70 eV) in split injection mode (1:20). The same column and temperature program as in GLC-FID was used. Data evaluation was performed using Amdis Analysis (Version 2.68).

Methylation of GM

The polysaccharide (Mannan, SGM or KGM) was dissolved in DMSO or water. In the aqueous system, the base and MeI were both added to the polysaccharide solution. When Li-dimsyl was used, the base was added first to the polysaccharide solution, and after 30 min (called the “incubation time” and not included in the "reaction time"), methyl iodide was added. The reaction conditions for the individual samples are shown in Table 1. Methylation of mannan and KGM were performed in H_2O using NaOH/MeI, while SGM was methylated in DMSO using Li-dimsyl/MeI. The reactions were carried out under nitrogen at room temperature (r.t.). Next, 1.6 M Li-dimsyl ($CH_3SOCH_2^-$ Li^+) was freshly prepared from methyl lithium (5% in diethyl ether) and dry DMSO under nitrogen.

Table 1 Methylation conditions of mannan and glucomannans. Concentration of polysaccharide: 10 mg/mL (KGM2: 25 mg/mL; mannan: 50 mg/mL); incubation time with Li-dimsyl: 30 min.; reactions were performed at room temperature.

Sample Name	Reagent (Base/ Solvent)	Base [equiv./ glycosyl unit]	CH_3I [equiv./ glycoyl unit]	Reaction time [h]
MeMan	NaOH/H_2O	2.0	2.20	48
MeKGM	NaOH/H_2O	1.5	2.25	24
MeSGM1	Li-dimsyl/DMSO	0.1	0.15	2
MeSGM2	Li-dimsyl/DMSO	0.3	0.45	2
MeSGM3	Li-dimsyl/DMSO	0.5	0.75	2
MeSGM4	Li-dimsyl/DMSO	0.8	1.20	2
MeSGM5	Li-dimsyl/DMSO	1.2	1.80	2
MeSGM6	Li-dimsyl/DMSO	3.0	4.50	1
MeSGM7	Li-dimsyl/DMSO	3.0	4.50	3
MeSGM8	Li-dimsyl/DMSO	12.0	18.00	3

The partially methylated samples were diluted and isolated using dialysis (molecular weight cut off, MWCO, 3500 Da), followed by freeze drying.

Preparation of Aldiol Acetates for Monomer analysis (AAM method)[15]

The partially methylated polysaccharide (ca. 2 mg) was hydrolyzed using 2 M trifluoroacetic acid (TFA) for 120 min at 120 °C in 1-mL V-vials. After cooling to r.t., the aqueous acid was removed under a stream of nitrogen until the residue was nearly dry. To neutralize any remaining traces of acid, a droplet of NaOH solution was added.

Subsequently, the monosaccharides obtained via hydrolysis were reduced to alditols. A solution of 0.5 mL of 0.25 M $NaBD_4$ in 2 M NH_3 (aq.) was added to the residue and heated at 60 °C for 120 min. After cooling to r.t., the residual $NaBD_4$ was quenched using acetic acid, and the borate was removed by repeated evaporation (5 times) with methanolic acetic acid (15%) under a stream of nitrogen.

Alditols were acetylated with 200 µL acetic anhydride and 50 µL pyridine at 90 °C for 120 min. After cooling to r.t., the solution was transferred into a 5-mL V-vial. The 1 mL V-vial was washed 3 times with dichloromethane (ca. 0.8 mL). The washing solutions were collected in 5-mL V-vials. The dichloromethane phase was washed once with saturated aq. $NaHCO_3$ (ca. 1.6 mL). After phase separation, the upper phase was carefully removed using a Pasteur pipette. The combined organic layers were washed once with 0.1 M HCl (1 mL), and subsequently washed three times with 1 mL of water. The organic phase was dried using $CaCl_2$ and used for GLC-FID analysis.

RESULTS AND DISCUSSION

The methylation of glucomannans leads to eight possible substitution patterns for each glucose and mannose moiety, which are classified as *non*-substituted (c_0), *mono*-substituted (c_1), *di*-substituted (c_2) and *tri*-substituted (c_3) units. In addition, up to tetra-*O*-methylated compounds can be obtained from the terminal residues, including the reaction of O-4. The position of the methylation can be deduced from the mass spectra (GLC-MS) of the alditol acetates prepared according to Fig. 2, but to distinguish stereoisomers, i.e., mannitols and glucitols, standards are required. These were prepared from mannan after partial methylation and from commercial methylcellulose (DS 2.02). GLC analysis of a partially methylated alditol acetate (PMAA) mixture of these two standards provides the retention times of all PMAAs of interest, which allowed unambiguous assignments of the peaks of MeSGM and evaluation of the methyl patterns (see Fig. 3).

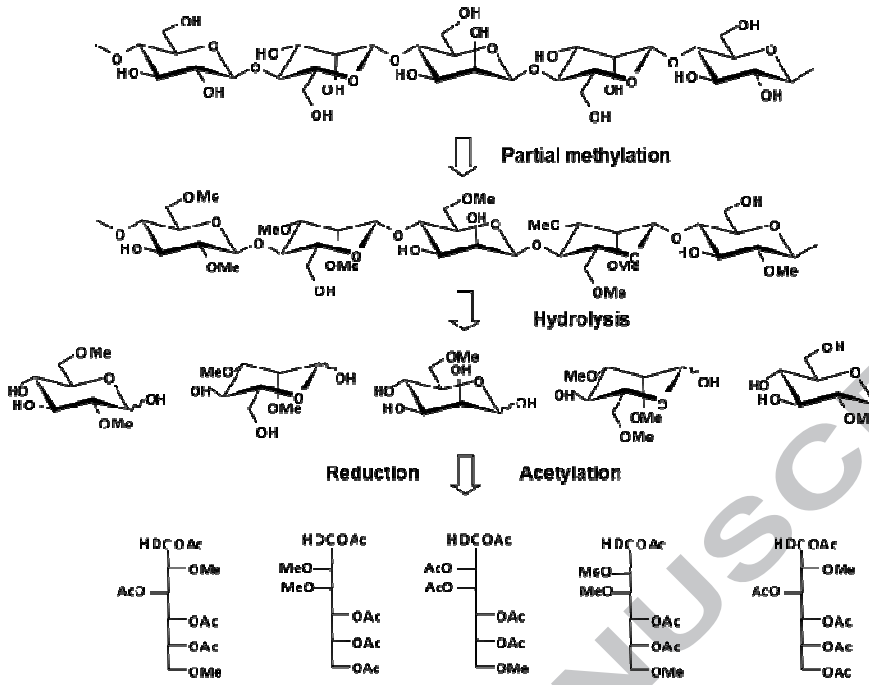

Fig. 2 Scheme of the methylation and preparation of the alditol acetates for the analysis of the methyl pattern of the GMs

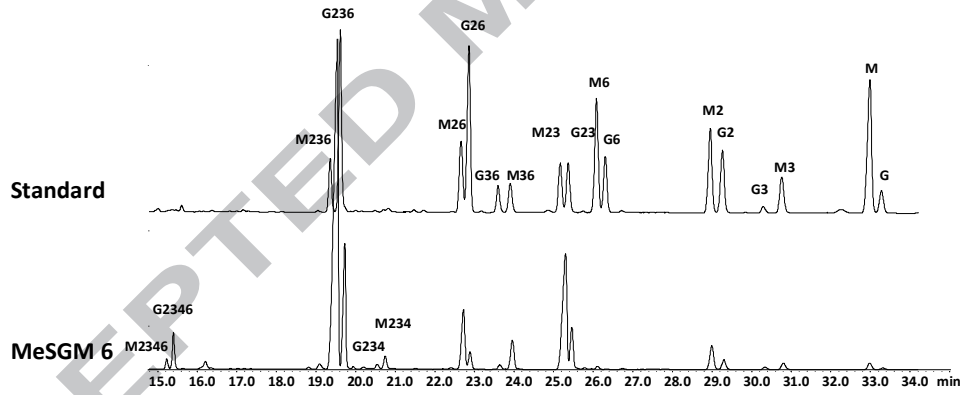

Fig. 3 Gas chromatograms of the standards (co-injection of alditol acetates from partially methylated mannan and methyl cellulose), alditol acetates of MeSGM 6 (DS 2.09). The peaks from glucose (G) and mannose (M) are assigned according to the methylated position.

The yield for methylated SGM was in the range of 87% to quantitative yield, while for methylated KGM the yield was 70-75%. The peak areas of all of the derivatives were corrected according to the effective carbon response concept [21]. The methylation analysis of SGM used in this study indicates that both mannose and glucose were involved in terminal and branched positions, corresponding to their mol fractions present.

The results for MeSGM 1 to MeSGM 8 are shown in Table 2. Due to the approximately 7.4% branching units in SGM, detailed values for the molar fractions for up to 20 constituents s_i,

including terminal 2,3,4,6- and 2,3,4-*O*-methylated units, are considered in the calculations. For the evaluation of the relative reactivities, 4-*O*-methylation was neglected. Therefore, the 2,3,4,6-tetra-*O*-methylated units were summarized with 2,3,6-tri-*O*-methylated units, and 2,3,4-tri-*O*-methylated were added to the mol fraction of 2,3-di-*O*-methylated residues. The molar fractions c_i of *i*-fold substituted glycosyl units, partial DS values x_i of the individual hydroxyl groups in position *i*, and total DS are listed in Table 2 as well. In SGM, the M/G ratio of the starting material was 3.8. For a better comparison, the molar compositions of mannose and glucose were individually normalized to 100%.

Table 2 Relative molar composition (Mol %) of glucose and mannose of SGM substituted at position *i* (s_i), of molar fraction in % of *i*-fold substituted monomer units (c_i), partial DS-values x_i for position *i*, and total DS. Data are independently normalized for both glucose and mannose. The ratio of mannose to glucose in the SGM used was 3.8. Each value except for MeSGM 6 is the average of two independent analyses.

	Composition in Mol%																	
Me in position	**MeSGM 1**		**MeSGM 2**		**MeSGM 3**		**MeSGM 4**		**MeSGM 5**		**MeSGM 6**		**MeSGM 7**		**MeSGM 8**		**MeKGM**	
	Man	**Glc**	**Man**	**Glc**	**Man**	**Glc**	**Man**	**Glc**	**Man**	**Glc**	**Man**	**Glc**	**Man**	**Glc**	**Man**	**Glc**	**Man**	**Glc**
s_0	98.55	96.62	85.95	83.32	80.00	77.84	49.31	40.42	22.78	17.98	4.20	4.15	0.87	1.02	0.64	0.90	75.10	72.40
s_2	0.48	0.45	3.91	5.77	5.10	6.99	9.80	17.49	12.43	18.31	8.48	11.29	3.23	5.14	0.06	0.16	7.93	11.96
s_3	0.44	2.04	6.39	7.78	7.86	9.49	17.40	16.97	17.55	13.71	6.51	4.11	0.96	1.60	0.06	0.05	4.76	2.36
s_6	0.22	0.13	2.57	1.85	3.32	2.33	4.50	2.09	4.97	1.99	2.47	0.86	0.34	0.24	0.08	0.13	9.29	10.23
s_{23}	0.03	0.06	0.35	0.38	1.06	1.31	9.17	12.50	20.06	24.61	28.08	31.40	20.64	21.37	7.41	6.96	0.70	0.58
s_{26}	0.08	0.12	0.30	0.28	0.75	0.71	2.01	2.51	4.39	5.19	7.75	7.36	7.51	8.23	0.48	0.40	1.17	2.05
s_{36}	0.08	0.39	0.39	0.40	1.13	0.72	3.20	1.91	6.84	3.51	8.49	2.93	3.95	2.24	0.33	0.23	0.69	0.22
s_{236}	0.11	0.20	0.14	0.22	0.79	0.60	4.60	6.12	10.97	14.71	34.01	37.90	62.50	60.15	90.94	91.17	0.34	0.21
c_0	98.55	96.62	85.95	83.32	80.00	77.84	49.31	40.42	22.78	17.98	4.20	4.15	0.87	1.02	0.64	0.90	75.10	72.40
c_1	1.15	2.62	12.87	15.40	16.27	18.81	31.71	36.55	34.96	34.01	17.46	16.26	4.53	6.99	0.20	0.34	21.99	24.54
c_2	0.19	0.57	1.04	1.06	2.94	2.74	14.38	16.92	31.29	33.31	44.32	41.69	32.10	31.84	8.22	7.59	2.56	2.85
c_3	0.11	0.20	0.14	0.22	0.79	0.60	4.60	6.12	10.97	14.71	34.01	37.90	62.50	60.15	90.94	91.17	0.34	0.21
x_2	0.01	0.01	0.05	0.07	0.08	0.10	0.26	0.39	0.48	0.63	0.78	0.88	0.94	0.95	0.99	0.99	0.10	0.15
x_3	0.01	0.03	0.07	0.09	0.11	0.12	0.34	0.37	0.55	0.57	0.77	0.76	0.88	0.85	0.99	0.98	0.07	0.03
x_6	0.00	0.01	0.03	0.03	0.06	0.04	0.14	0.13	0.27	0.25	0.53	0.49	0.74	0.71	0.92	0.92	0.11	0.13
M/G ratio	4.03		4.12		4.02		4.09		3.78		3.75		3.99		3.81		1.67	
DS(M) and DS(G)	0.02	0.04	0.15	0.18	0.25	0.26	0.74	0.89	1.30	1.45	2.08	2.13	2.56	2.51	2.89	2.89	0.28	0.31
DS (SGM) Ø	0.02		0.16		0.25		0.77		1.33		2.09		2.55		2.89		0.29	

The DS values for O-2, O-3, and O-6 were between 0.02 and 2.89. MeSGM 8 is practically permethylated (total DS including O-4 is 3.01), and the corresponding alditol acetates reflect the branching pattern. From the data in Table 2, it is obvious that there is no difference in the distribution of glucose and mannose with respect to the terminal units and branching in SGM. Comparing the glucose and the mannose fractions, the individual DS of glucose is slightly higher, up to a DS of approximately 1.5, but at about DS2, mannose is equal to glucose. From the substitution patterns of the individual MeSGM (Table 2), it is evident that, in contrast to the typical methyl celluloses, the reactivity of the primary OH at C-6 is remarkably low. Substitution at the secondary OH groups is strongly favored with the highest reactivity at 3-OH. In mannose, 2-OH is equal to 3-OH at a DS of approximately 2 (MeSGM 6), while for glucose, it equals 3-OH at DS 0.8 (DS_{glc} 0.89, MeSGM 4). In commercial methylcellulose prepared in aqueous systems with NaOH and methyl chloride, typically 20-25% of methyl groups are located at O-3, 40% at O-2 and 35-40% at O-6. No direct influence of primary substitution on the reactivity of the remaining OHs in the glucosyl unit is observed for these derivatives [15].

The statistical model for a random substituent pattern, introduced by Spurlin for kinetically controlled reactions of cellulose, [22] was applied to compare the analytical data with the random pattern calculated for the partial DS values (x_i).

Fig. 4 shows the un-, mono-, di- and trisubstituted mol fractions (c_i) of the consecutive methylation reaction of glucose and mannose in SGM. Deviations from the Spurlin model are significant. As is typical for non-random distributions, the mol fractions of un- (c_0) and trisubstituted residues (c_3) are still or already higher than expected, while c_1 (mannose) or c_1 and c_2 (glucose) are lower. Obviously, methylation was favored in already methylated units, which indicates fast fluctuation of the protons in the partially deprotonated chains, because the electrostatic repulsion of anions suggests that the opposite effect would be expected. It will be of interest to look into the methylation patterns at the polymer level of SGM, which should be the result of the individual reactivities and the distribution of glucose and mannose in the GM macromolecules.

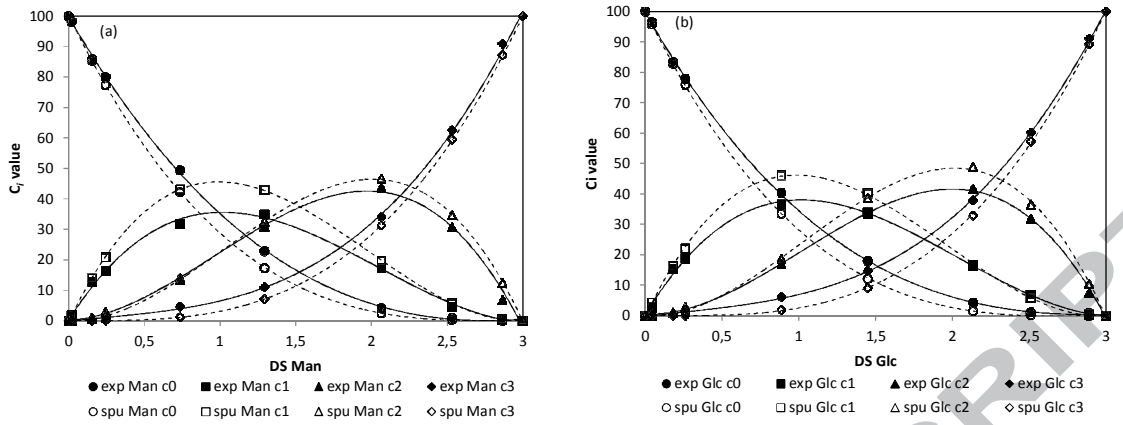

Fig. 4 Comparison of the experimentally determined mol fractions (c_i) for (a) Man and (b) Glc of MeSGM1-8 with model data for a random pattern

Methylation of carbohydrates can be performed in different ways. Typically, OH groups are deprotonated to form good nucleophiles, which then attack methyl halide (or dimethylsulfate) to form methyl ethers. This Williamson etherification is irreversible and, thus, kinetically controlled. However, when sub-stoichiometric amounts of base are applied, the degree of deprotonation will be different for the various OH groups at positions 2, 3 and 6 in the 1,4-glycan and between glucose and mannose. Because the acid-base reaction is reversible, this step is thermodynamically controlled, and the equilibria between ROH/RO^- are determined by their pK_a values. Thus, the regioselectivity of etherification will, to a certain extent, also reflect the relative acidities of the OH groups. In addition to the acidity, the stereoelectronic and steric effects have to be considered. Cyclodextrins, for example, show a much higher regioselectivity in favor of O-2 compared to the corresponding open-chain malto-oligosaccharides and amylose due to the poor accessibility of O-3 [18, 23]. Regarding the mechanism, the steric requirements of the transition state are higher for S_N2 than S_N1 reactions, as has been studied in reactions of selectively protected cycloinositols [24, 25]. Consequently, the solvent is an influential factor, especially in the case of substrates with reactive groups in close proximity. Because of the multifunctionality of carbohydrates, derivatization comprises several consecutive steps. For the data on MeKGM in aqueous reactions (Table 2), the overall reactivity is also slightly higher for glucose (MeKGM: DS(man) 0.28, DS (glc) 0.31). Different regioselectivities are found for glucose and mannose. For mannose, a slight preference for OH at position 6 is observed, while glucose shows more pronounced differences of reactivities following the order $2 \geq 6 > 3$, again an indication of the higher acidity of the equatorial 2-OH compared to the axial one. Thus, methylation of the primary 6-OH is much more competitive under these conditions and can be further enhanced by increasing the amount of base. According to the above mentioned model of Spurlin [22] for reactions of cellulose in aqueous systems, the relative reactivities of the

different OHs should remain constant over the course of the reaction as long as the glycosyl units in the polysaccharide are equally accessible. Changes in the primary substitution do not influence the residual OH groups, indicating that no neighboring groups influence the local reactivities.

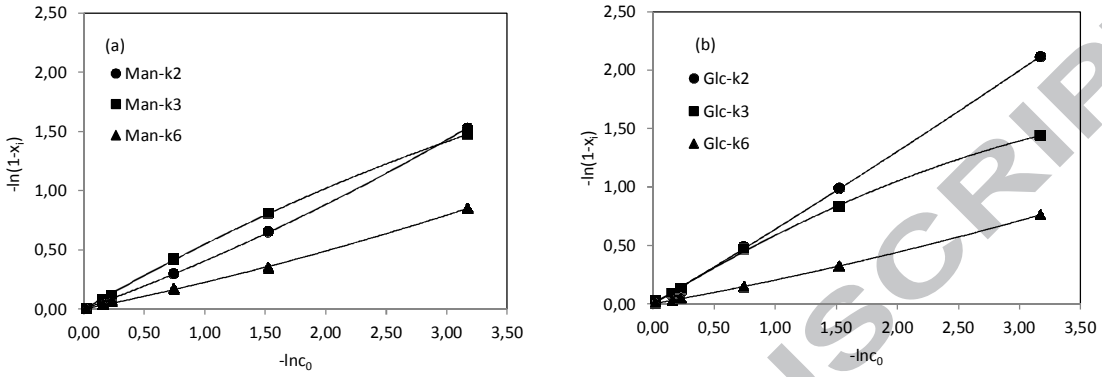

Fig. 5 Relative rate constants k_2, k_3 and k_6 calculated independently for both (a) Man and (b) Glc and obtained from the data on MeSGM1-6

If these requirements are fulfilled, a logarithmic plot, as shown in Fig. 5, should give a linear relationship with a slope indicating the relative reactivity constants, k_i. Instead of time, the decrease in unsubstituted glycosyl units, -ln c_0, is taken to be the reaction coordinate. We applied this evaluation to the MeSGM series. Figs. 5a and b show the results for mannose and glucose. To allow comparison of glucose and mannose, the same reaction coordinate must be used. Therefore, the -ln c_o of glucose and mannose was not used; instead, the c_o of MeSGM was used. As mentioned above, O-3 was the most reactive in the early stages of the reaction for both glucose and mannose. However, for glucose, the relative reactivities followed the order 2 > 3 > 6, while for mannose, the reactivity of O-3 was the highest over a wide portion of the reaction, closely followed by O-2. The reactivity of the primary 6-OH was far behind for both sugars, which is a distinct difference to reactions in water, where methylation of the primary OH, which has better accessible and is more acidic than the secondary 3-OH, is usually much more pronounced, as mentioned above. However, constant slopes were not observed for the individual OHs, but the data points could be fitted very well with an exponential.

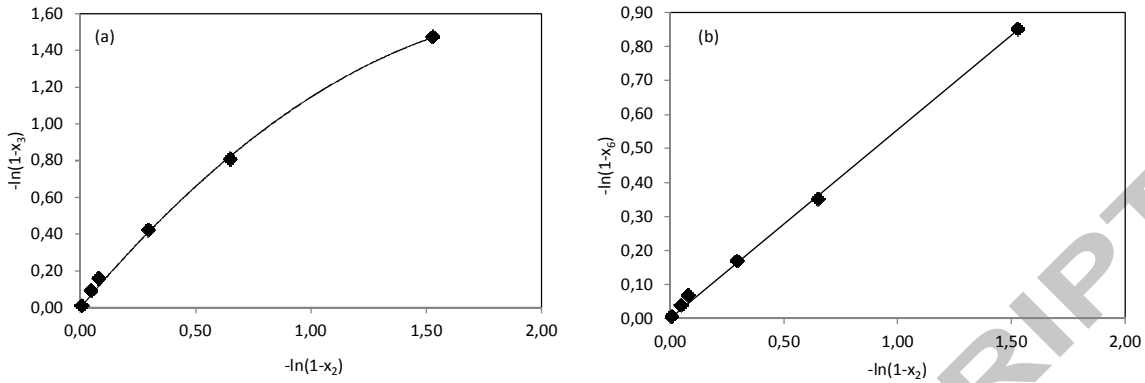

Fig. 6 Relationship of relative reactivities in mannose of MeSGM 1-6: (a) for O-2 and O-3 (b) O-2 and O-6.

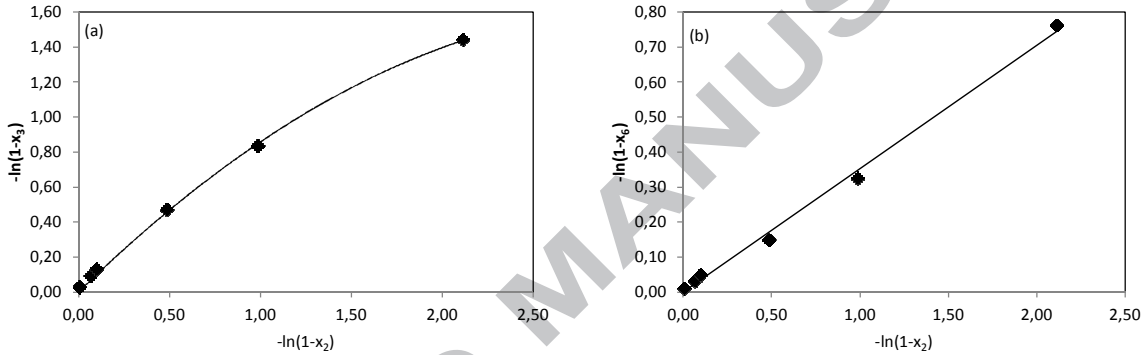

Fig. 7 Relationship of the relative reactivities in glucose of MeSGM 1-6: (a) for O-2 and O-3 (b) O-2 and O-6.

The relative reactivity of O-3 decreased with increasing methylation of O-2 (see Fig. 6a for mannose and Fig. 7a for glucose), while O-6 remained unaffected by the status of O-2 (Fig. 6b for mannose and Fig. 7b for glucose). This result is observed for both mannose and glucose. MeSGM1-6 were included in these calculations because the higher partial DS x_i approaches the maximum value of 1, and the uncertainties in the $-\ln(1-x_i)$ values are higher.

What is the reason for this completely different reactivity and change in the relative reactivities in DMSO compared to water? Reactions with weak and strong bases, in aqueous and aprotic systems, have to be differentiated. In the carbohydrate methylation reactions, which take place in an aqueous system, the polysaccharides are dissolved or swollen and activated in the NaOH solution and should thus be equally accessible [24]. The presence of base and methyl halide is possible because the HO^- is not nucleophilic enough that it consumes all of the alkylating agent. Due to very good solvation of the anions, the reactive species are well shielded and not influenced by their neighboring groups. Therefore, they react independently and show the distribution calculated for consecutive reactions with a constant ratio of reactivities of 2-, 3- and 6-OH, which is also supported by the low

temporary concentration of alcoholate groups. Deprotonated hydroxyls can immediately react with the electrophile.

In contrast, the alkylation with the stronger base, Li-dimsyl (pK_a DMSO 35), needs to be performed in two steps [26]. SGM was dissolved in DMSO. The calculated amount of Li-dimsyl solution was added (see Table 1) and consumed for deprotonation of the polysaccharide before the alkyl halide was added, which is necessary to avoid alkylation of DMSO.

The alcoholic OH of the carbohydrates is more acidic than common aliphatic mono-alcohols with the hydroxyl at the C-2 next to the electron-withdrawing anomeric center being the most acidic (pK_a ca. 13). Axial OH are known to be less acidic then equatorial OH [27]. They also have less electron-withdrawing effects than the equatorial groups [28].

Because the anions formed in the deprotonation step are not solvated by the aprotic DMSO, they "help themselves" by sharing the proton of the vicinal OH. The most acidic OH at C-2 is preferentially deprotonated and forms an intramolecular H-bridge with 3-OH [27], see Fig. 8.

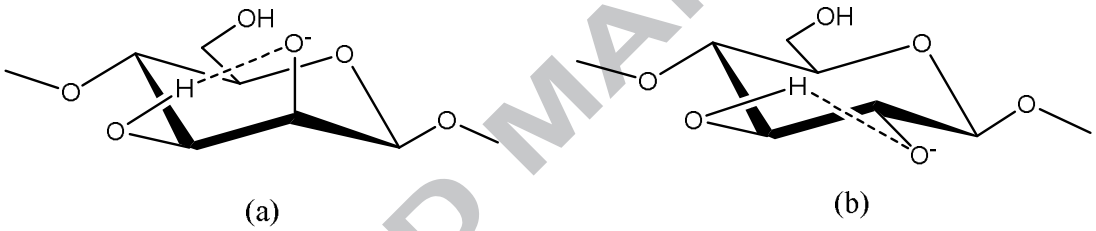

Fig. 8 The intramolecular H-bridge formed in (a) Man and (b) Glc after deprotonation at O-2 in an aprotic solvent

When the alkylating agent is added, the deprotonation pattern is probably not simply trapped by methyl iodide. The regioselectivity of etherification is also influenced by steric factors. Thus, the more accessible equatorial 3-OH benefit from the interaction with the neighboring, more acidic 2-OH. As illustrated in Fig. 8, in the aprotic solvent the naked RO^- ion at C-2 will interact with 3-OH, thus sharing the proton and activating O-3 for a nucleophilic attack. This effect is more pronounced for mannose than for glucose because of the axial orientation of 2-OH in the earlier. Due to the ecliptic position of H-4 and the interference with the non-binding electron pairs at the glycosidic oxygens with the axial O-2, the nucleophilic reaction of the equatorial O-3 is much more favored than is expected from its acidity. As the methylation of O-2 increases, this "catalytic effect" disappears, and the relative reactivity of O-3 decreases.

CONCLUSION

The relative reactivities of stereoisomeric glucose and mannose in glucomannan (SGM and KGM) were studied. MeKGM with a DS of 0.3 from the reaction in aqueous solution with NaOH/MeI showed the order O-6 > O-2 > O-3 with only slight differences, while for glucose, the reactivities of O-2 and O-6 were both distinctly more reactive than O-3. A series of MeSGM was prepared in DMSO using soluble Li-dimsyl/MeI to obtain the relative reactivities of the OH groups in stereoisomeric glucose and mannose. The results from this homogeneous reaction clearly showed the preference for the equatorial 2-OH in glucose, followed by 3-OH and 6-OH for glucose, while for mannose, the reactivity of 3-OH was the highest up to DS 2, and at this DS, it was matched by O-2. The reactivity of the primary 6-OH was the lowest for both epimeric sugars. In the aprotic solvent, the less acidic 3-OH most likely benefited from the interaction with the anion formed from the more acidic 2-OH. The equatorial position of 2-OH in glucose favors its reactivity over the axial 2-OH in mannose by a factor of 1.4 because it is more acidic and less sterically hindered. Comparing the relative reactivities of 2-OH and 3-OH, the latter can compete with the 2-OH in mannose. In conclusion, up to a DS of approximately 2, the order of the six OH groups in GM is G-k_2 > M-k_3 ≥ M-k_2 > G-k_3 > M-k_6 >G-k_6.

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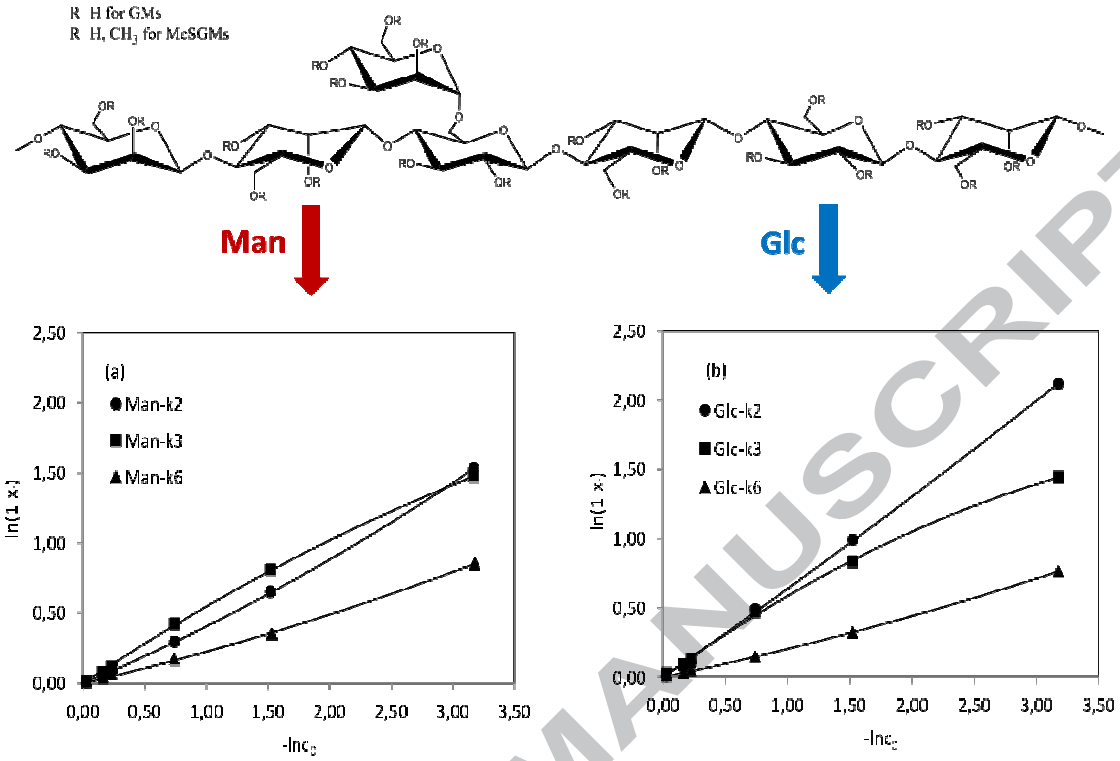
RELATIVE REACTIVITIES IN THE O-METHYLATION OF GLUCOMANNANS: THE INFLUENCE OF STEREOCHEMISTRY AT C-2 AND THE SOLVENT EFFECT

Figure legends

1. **Fig. 1** Proposed structures for spruce glucomannan and its partially methylated derivatives. Approximately 20% of the units have the glucosyl-configuration, and 80% have the mannosyl configuration.
2. **Fig. 2** Scheme of the methylation and preparation of the alditol acetates for the analysis of the methyl pattern of the GMs
3. **Fig. 3** Gas chromatograms of the standards (co-injection of alditol acetates from partially methylated mannan and methyl cellulose), alditol acetates of MeSGM 6 (DS 2.09). The peaks from glucose (G) and mannose (M) are assigned according to the methylated position.
4. **Fig. 4** Comparison of the experimentally determined mol fractions (c_i) for (a) Man and (b) Glc of MeSGM1-8 with model data for a random pattern
5. **Fig. 5** Relative rate constants k_2, k_3 and k_6 calculated independently for both (a)Man and (b)Glc and obtained from the data on MeSGM1-6
6. **Fig. 6** Relationship of relative reactivities in mannose of MeSGM 1-6: (a) for O-2 and O-3 (b) O-2 and O-6.
7. **Fig. 7** Relationship of the relative reactivities in glucose of MeSGM 1-6: (a) for O-2 and O-3 (b) O-2 and O-6.
8. **Fig. 8** The intramolecular H-bridge formed in (a) Man and (b) Glc after deprotonation at O-2 in an aprotic solvent

Graphical abstract



Highlights

- A set of *O*-methyl glucomannans (GM) in DS range 0.02-2.89 were prepared in DMSO with Li-dimsyl/methyl iodide
- Relative reactivities of stereoisomeric glucose and mannose in glucomannan were studied
- Up to DS 2, the order of the six OH groups in GM is G-k_2 > M-k_3 ≥ M-k_2 > G-k_3 > M-k_6 >G-k_6.
- Enhanced reactivity of O-3 in both glucose an mannose in the early stage of the reaction is explained by a solvent effect.