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Targeted allylation and propargylation of galactose-containing polysaccharides in water

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ABSTRACT

Galactose units of spruce galactoglucomannan (GGM), guar galactomannan (GM), and tamarind (galacto)xyloglucan (XG) were selectively allylated. Firstly aldehyde functionalities were formed at the C-6 position via enzymatic oxidation by galactose oxidase. The formed aldehydes were further derivatized by an indium mediated Barbier–Grignard type reaction, resulting in the formation of homoallylic alcohols. In addition to allylic halides, the same reaction procedure was also applicable for GGM, when using propargyl bromide as halide. All reaction steps were done in water, thus the polysaccharides were modified in a one-pot reaction. The formation of the allylated, or propargylated, product was identified by MALDI-TOF–MS. All polysaccharide products were isolated and further characterized by GC–MS or NMR spectroscopy. By this chemo-enzymatic process, we have demonstrated a novel method for derivatization of GGM and other galactose-containing polysaccharides. The derivatized polysaccharides are potential platforms for further functionalizations.

1. Introduction

Naturally occurring non-cellulosic polysaccharides, such as wood and plant hemicelluloses, are becoming increasingly interesting as starting materials in the search for environmentally sustainable industrial processes. Polysaccharides are already in use, for example, in the food and cosmetic industries, but new application areas are enabled as novel plausible methods for polysaccharide modifications are developed. Desired physico-chemical properties, such as solubility or hydrophobicity, of polysaccharides can often be reached by introducing functional groups to the polysaccharides (Cunha & Gandini, 2010a, 2010b; Xu et al., 2010, 2011).

An interesting approach to chemical modification of polysaccharides is the introduction of highly reactive carbon–carbon double or triple bonds. Both functional groups can be subsequently reacted enabling anchoring of desired functionalities to the polysaccharide, i.e. stimuli-responsive moieties, hydrophobic tails, bioactive, and UV-sensitive compounds. These may find applications in biomedical, food, intelligent packaging, and bio-detection applications (Maharjan et al., 2008; Cheng et al., 2010). The double bonds in allylated starch have been used to form epoxides (Huijbrechts et al., 2010) and for co-polymerization with acrylic monomers (Bhuniya, Rahman Md, Satyanand, Gharia, & Dave, 2003), while the triple bonds of propargylated starch have been further reacted using "click-chemistry" (Elchinger et al., 2011; Tankam, Müller, Mischnick, & Hopf, 2007).

The allylation of carbohydrates was first reported by Tomecko and Adams (1923). In order to introduce reactive groups to polysaccharides, the authors formed allyl ethers of e.g. dextrin, starch, and cellulose by reacting the polysaccharides with allyl bromide in alkaline conditions. Allyl ethers of polysaccharides have also been prepared using allyl glycidyl ether (Ameye et al., 2002; Duanmu, Gamstedt, & Rosling, 2007; Huijbrechts et al., 2007) and in different solvent systems, such as *N,N*-dimethylacetamide and lithium chloride (LiCl/DMAc) (Lin & Huang, 1992) or dimethyl sulfoxide/tetrabutylammonium fluoride trihydrate (Heinze, Lincke, Fenn, & Koschella, 2008).

Aldehydes and ketones can be allylated using a Barbier–Grignard type reaction, where the carbonyl group

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reacts with an allyl halide in the presence of a metal mediator to form a homoallyl alcohol (Pétrier & Luche, 1985; Li, 1996). In carbohydrate chemistry, this reaction has previously been applied on unprotected monosaccharides to extend aldoses, and in synthesis of bioactive compounds (Balla, Zamyatina, Hofinger, & Kosma, 2007; Chan & Li, 1992; Gao, Martichonok, & Whitesides, 1996; Gordon & Whitesides, 1993; Schmid & Whitesides, 1991). We have recently allylated the C-6 of methyl galactopyranoside through combining enzymatic oxidation by galactose oxidase (GO, E.C. 1.1.3.9) with indium mediated allylation (Leppänen et al., 2010). Since both the enzymatic oxidation and the metal mediated allylation reaction are performed in water, it is possible to obtain allylated polysaccharides in a one-pot reaction. In addition, the same indium mediated procedure could also be applied on propargyl bromide to introduce alkynyl groups to the polysaccharide. The enzyme GO catalyses the oxidation of primary alcohols to corresponding aldehydes (Whittaker, 2003). GO is regioselective towards the hydroxyl at C-6 of galactose units, and it can, in combination with catalase and horse radish peroxidase (HPR), be used for selective oxidation of galactopyranosyl units in polysaccharides (Hartmans et al., 2004; Parikka et al., 2010). Polysaccharides that thus can be selectively oxidized by GO are, for example, spruce *O*-acetyl-galactoglucomannan (GGM), guar galactomannan (GM), and (galacto)xyloglucan (XG). Especially the use of GGM is of interest for the Nordic forest industry due to the availability of the polysaccharide in pulping of softwood and in particular, spruce.

We present here a novel method for introduction of allyl groups to polysaccharides. The galactose-containing polysaccharides GGM, GM, and XG were successfully allylated using enzymatic oxidation combined with indium mediated allylation. Reactions with allyl bromide, crotyl chloride, and cinnamyl chloride were assessed for the introduction of different functional groups. In addition, the same procedure was applicable when performing reactions using propargyl bromide. The reaction products were analyzed by a MALDI–MS method, by which the approximate conversion to reaction products could feasibly be observed. This is the first report on the utilization of MALDI–MS in the analysis of enzymatically and chemically modified GGM, and GM derivatives.

2. Materials and methods

2.1. Materials

GGM was prepared from spruce thermomechanical pulp (TMP) by a large laboratory-scale method modified from the method reported by Willför, Rehn, Sundberg, Sundberg, & Holmbom (2003). In short, a suspension of TMP in hot tap water was stirred for 3 h and the pulp was removed. The extract water was purified from colloidal wood resin, and aromatic residues using a cationic coagulant (Raifix 120, Raisio Chemicals Oy, Finland) and XAD-7 resin (Amberlite, Rohm and Haas, UK). The water was concentrated by ultra-filtration before GGM was isolated by precipitation in ethanol and air dried.

XG of reduced molar mass (M_w 1.7×10^4 g/mol, M_w/M_n 2.0) was prepared from tamarind seed xyloglucan (Innovasynth Technologies Ltd., India) by digestion with the xyloglucan-specific endo-glucanase (EGase, EC 3.2.1.151, specific activity 70,680 U/25 g) from *Chrysosporium lucknowense* (xgl1). EGase was purchased from Dyadic NL, Wageningen, NL.

Raffinose and GM were purchased from Sigma–Aldrich, and were used without further purification.

Galactose oxidase (GO) for oxidation of GGM and GM was a gift from Hercules (Barneweld, Netherlands). It was produced by *Pichia pastoris* carrying the gene encoding GO from *Fusarium* spp., and used without further purification. As the activity of GO was not known, the reported specific activity of a similar preparation was used for the estimation of the GO amounts (26 U/mg). For the oxidation of XG, GO from *Fusarium graminearum* was produced recombinantly in *Pichia pastoris* as described previously (Spadiut, Olsson, & Brumer, 2010). Horseradish peroxidase (P8250, Type II, 181 U/mg) and catalase (C30, from bovine liver, 22,000 U/mg) were purchased from Aldrich. Endo-1,4-β-mannanase (1,4-β-D-mannan mannanohydrolase) (*Cellvibrio japonicus*), EC 3.2.1.78 (420 U/mg at 40 °C, pH 7) was purchased from Megazyme.

All chemicals were of commercial grade. Allyl bromide was purchased from Merck (Hohenbrunn, Germany), crotyl chloride, cinnamyl chloride and propargyl bromide (80 wt.% in toluene) from Sigma–Aldrich (Steinheim, Germany). Indium powder (100 mesh) was purchased from Sigma–Aldrich (Steinheim, Germany).

2.2. Experimental methods

2.2.1. Enzymatic oxidation

The polysaccharides were enzymatically oxidized using galactose oxidase in combination with horseradish peroxidase (HRP) and catalase, as previously reported by Parikka and Tenkanen (2009) and Parikka et al. (2010). In short, the polysaccharide was dissolved in water (1–10 mg/mL), and the enzymes GO, catalase, and HRP were added. The amount of GO was related to the approximate amount of terminal galactose present in the polymer (0.052 U of GO/1 mg of galactose). The dosage of catalase was 115 U/mg and HRP 1.5 U/mg. The solutions were stirred at room temperature for 48 h, and were then heated in boiling water for ca. 10 min to inactivate the enzymes. The oxidized polysaccharides were not isolated; the indium mediated allylations were performed directly in the solutions in which the oxidations were done.

2.2.2. General procedure for indium-mediated allylation and propargylation

The amounts of reactants were related to the approximate molar amount of oxidized galactose units in the solution. Plain water was used as solvent in most reactions. Allylation reactions of oxidized raffinose were also done using methanol (MeOH) or tetrahydrofuran (THF) as co-solvents (co-solvent-H_2O 1:4, v/v). Oxidized GGM was also allylated in HCl (0.1 M), or using THF as co-solvent (THF-H_2O 1:4, v/v).

The typical amount of sample was ca 10 mg. To the aqueous solution containing the oxidized polysaccharide (1–10 mg/mL), indium powder (5–10 equiv.) and the allylic or propargylic halide (5–10 equiv.) were added, and the solution was stirred at room temperature or at 55 °C for 24–48 h. The allylated polysaccharide was isolated by precipitation in ethanol (ethanol-water 10:1, v/v), washed twice with ethanol–water (10:1, v/v), and dried by freeze-drying or under N_2.

2.2.3. Indium-mediated allylation of oxidized GGM

To the solution of oxidized GGM in water (~6 mg/mL, 25 mL), indium powder (60 mg) and allyl bromide (60 mg) were added, and the solution was stirred at 55 °C for 24 h. The allylated GGM was then filtered through a glass microfiber filter to remove the indium before it was isolated by precipitation in ethanol (ethanol–water 10:1, v/v), washed twice with ethanol–water (10:1, v/v), and dried by under N_2 and in a vacuum desiccator. The product was obtained in 54% yield as a white powder.

2.3. Analytical methods

2.3.1. The carbohydrate content

The carbohydrate content of the modified polysaccharides was determined by gas chromatography (GC) and by gas

chromatography and mass spectroscopy (GC–MS) after methanolysis and silylation (Willför et al., 2009). GC analysis was done on a PerkinElmer AutoSystemXL instrument (Norwalk, USA) equipped with an HP-1 column. The temperature program used was: 100–175 °C, 4°/min, 175–290 °C, 12°/min. Injector 260 °C, detector 290 °C. GC–MS was done on an HP 6890-5973 GC-MSD instrument equipped with an HP-1 column. The temperature program used was: 80 °C (0.5 min) – 300 °C, at 8 °C/min.

2.3.2. The degree of oxidation

The degree of oxidation (DO), i.e. the amount of galactose units containing an aldehyde group (Gal-CHO) relative to the total amount of galactose units, of modified GGM's and GM's was determined by deuterium labelling of the aldehydes by reduction with $NaBD_4$. After acid methanolysis and silylation, the DO could be determined from the GC-MS spectra by comparing the abundance of the peaks at *m*/*z* 361 and *m*/*z* 362 (Parikka et al., 2010). The DO is defined as:

$$\mathrm{DO}\,(\%) = \left(\frac{[\text{Gal-CHO}]}{([\text{Gal-CHO}] + [\text{Gal}])}\right) \times 100 \quad (1)$$

where [Gal-CHO] is the amount of oxidized galactose units, and [Gal] is the amount of unoxidized galactose units.

DO of oxidized XG was determined by deuterium labelling of the aldehydes by reduction with $NaBD_4$ (Xu et al., 2012; Parikka et al., 2012). Afterwards, the product was digested with EGase as follows: To a 1 mL aqueous solution of xyloglucan (1 mg) 20 μL of 1 M solution of sodium acetate buffer (pH = 4.5) was added. The sample was then incubated with EGase (50 μg) at 37 °C for two hours. Following electrospray ionization mass spectrometry (ESI–MS), the level of galactose oxidation was approximated by the peak height of the monoisotopic ion peaks for the protonated and deuterated isomers.

ESI-MS was performed on a Micromass Q-Tof II (Waters Corp., Micromass MS technologies, Manchester, UK) in positive ion mode from an aqueous solution containing 0.5 mM NaCl.

2.3.3. Structural characterization

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF–MS) was performed with a SAI LT3 Plus MALDI-TOF mass spectrometer (SAI Ltd., Manchester, UK) equipped with a nitrogen laser of 337 nm and operated in positive ion mode (Xu et al., 2012). MALDI-TOF–MS samples were prepared by mixing 0.5 μL of sample solution with 0.5 μL of matrix solution (10 mg/mL 2,5-dihydroxybenzoic acid in acetone) on the MALDI-TOF-plate followed by drying under a stream of air. The polysaccharides were enzymatically digested prior to the analysis; GGM and GM by endo-1,4-β-mannanase, and XG by with EGase. Raffinose was analyzed directly without any pre-treatment.

NMR spectra of the isolated allylation products were recorded on a Bruker AV 600 instrument. A mixture of DMSO-d_6 and D_2O (1:2) was used as solvent.

3. Results and discussion

Raffinose and the galactose-containing polysaccharides GGM, GM, and XG, were derivatized by combining enzymatic oxidation and an indium mediated allylation reaction, as is discussed later. The formation of the desired homoallylic alcohols was verified by MALDI–MS analysis. The polysaccharides require partial enzymatic hydrolysis to oligosaccharides prior to analysis, thus raffinose, a trisaccharide containing an α-(1 → 6)-linked galactose group (Fig. 1), was chosen as a model compound resembling the formed oligosaccharides. Because of the small size of raffinose, MALDI–MS analysis could be done directly on the reaction solution, making the optimization of reaction parameters easier and faster.

In addition to MALDI–MS, also GC–MS was used for monitoring the reactions. The decrease in DO gave an indication of the formation of allylated, or propargylated, products, even if some of the changes could be assigned to some side reactions occurring simultaneously to the allylation reactions. The conversions of the allylation reactions, i.e. the decrease in the amount of Gal-CHO, were calculated from the decrease in DO using the following formula:

$$\text{Conversion}\,(\%) = 1 - \frac{(\mathrm{DO}_2 \times (1 - \mathrm{DO}_1))}{(\mathrm{DO}_1 \times (1 - \mathrm{DO}_2))} \quad (2)$$

where DO_1 is the degree of oxidation before the reaction, and DO_2 after the reaction. In the case of XG, the amount of galactose detected after reduction by $NaBD_4$ was assumed to originate from unreacted Gal-CHO units, and the conversions were calculated directly from those values.

3.1. Enzymatic oxidation

Raffinose, GGM, GM, and XG were activated for further chemical modifications through enzymatic oxidation. In GGM, approx. 10%, and in GM approx. 40%, of the sugar units are α-D-galactopyranosyl units attached to the mannopyranosyl units of the backbone through (1 → 6) linkages (Wielinga, 2000; Willför, Sjöholm, et al., 2003). In XG, approx. 16% of the sugar units are D-galactopyranosyl residues linked through (1 → 2) bonds to the xylose side chains, attached to the glucose backbone (York, Harvey, Guillen, Albersheim, & Darvill, 1993) (Fig. 1). The C-6 of these terminal galactopyranosyl units were oxidized using GO in combination with HRP and catalase. Reactive aldehyde groups (Gal-CHO) were successfully formed as has previously been reported by Parikka and Tenkanen (2009) and Parikka et al. (2010). The DO, determined by GC-MS after reduction of the oxidized galactose units with $NaBD_4$, was for GGM 60% (corresponding to approx. 6% of total sugars), and for GM 80% (approx. 32% of total sugars). XG was oxidized completely, that is, all terminal galactose units were in the corresponding aldehyde form.

The presence of hemiacetal bonds between hydroxyl and aldehyde groups decreases the solubility of the enzymatically oxidized polysaccharides making re-dissolution after drying difficult. To avoid this, the subsequent allylation and propargylation reactions were performed in the same solutions as the enzymatic oxidation.

3.2. Indium mediated allylation and propargylation

Metal mediated allylation of carbohydrates have previously been done using tin and indium as mediating metals (Kim, Gordon, Schmid, & Whitesides, 1993; Schmid & Whitesides, 1991). Indium has been shown to be the most efficient mediating metal, and indium mediated reactions produce fewer by-products than tin mediated ones (Kim et al., 1993), and indium was thus chosen in our experiments. The halides allyl bromide, cinnamyl chloride, crotyl chloride, and propargyl bromide were chosen as model substances due to their commercial availability. The enzymatically formed aldehyde groups in raffinose, GGM, GM, and XG were further chemically derivatized by the indium mediated allylation reaction (Scheme 1). The reaction was verified by MALDI–MS. Even if the analysis was not quantitative, the spectra still gave indications of the reaction efficiency; the existence of desired oligomers proved the reaction, the lack of starting material indicated a complete reaction (high efficiency), and the existence of oligomers originating from the starting material indicated an incomplete reaction (moderate or poor efficiency) (Table 1).

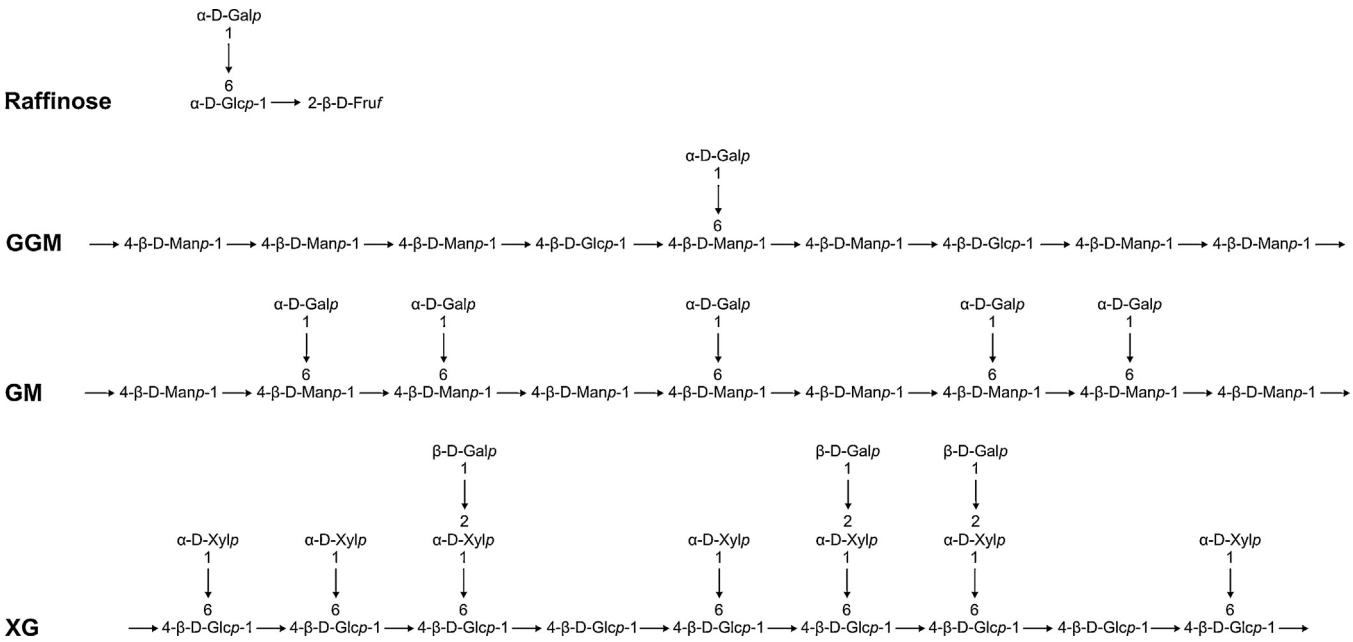
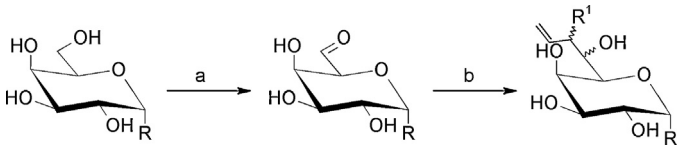


Fig. 1. The structures of raffinose, GGM, GM, and XG.



Scheme 1. Enzymatic oxidation of galactopyranosyl units followed by indium mediated allylation. (a) Galactose oxidase (GO), horseradish peroxidase (HRP), catalase, H_2O, room temperature and (b) indium, H_2O, 0 or 55 °C. R = polysaccharide backbone; R^1 = H, Me, or Ph; X = Br or Cl.

3.2.1. Allylation and propargylation of oxidized raffinose

All reactions were done in plain water. The reactions between enzymatically oxidized raffinose (Raf-CHO) and allyl bromide or cinnamyl chloride proceeded at room temperature. According to MALDI–MS analysis of the reaction solution, the reaction with allyl bromide was complete after 24 h, and since only the expected product peaks of allylated raffinose (m/z = 567, Na^+ adduct; m/z = 583, K^+ adduct) were detected (Fig. 2 and Table S1 in supplementary data), the reaction was assumed to have a yield of 100%. This was well in accordance with the results obtained

Table 1
Summary of indium mediated allylations and propargylations.

Oxidized sugar	Halide[a]	Reaction conditions[b]	Reaction efficiency[c]
Raffinose	Allyl	A	+++
Raffinose	Prop	B (A)	+++(0)
Raffinose	Cinn	A	+
Raffinose	Crot	A/B	0
GGM	Allyl	B	+++
GGM	Prop	B/C	+
GGM	Cinn	B	+
GGM	Crot	B	0
GM	Allyl	B	+
GM	Cinn	B	0
XG	Allyl	B	+++
XG	Prop	B	0
XG	Cinn	B	+

[a] Allyl = allyl bromide; Prop = propargyl bromide; Cinn = cinnamyl chloride; Crot = crotyl chloride.
[b] A = room temperature, H_2O; B = 55 °C, H_2O; C = 55 °C THF-H_2O (1:4).
[c] +++ = high; + = moderate; 0 = poor.

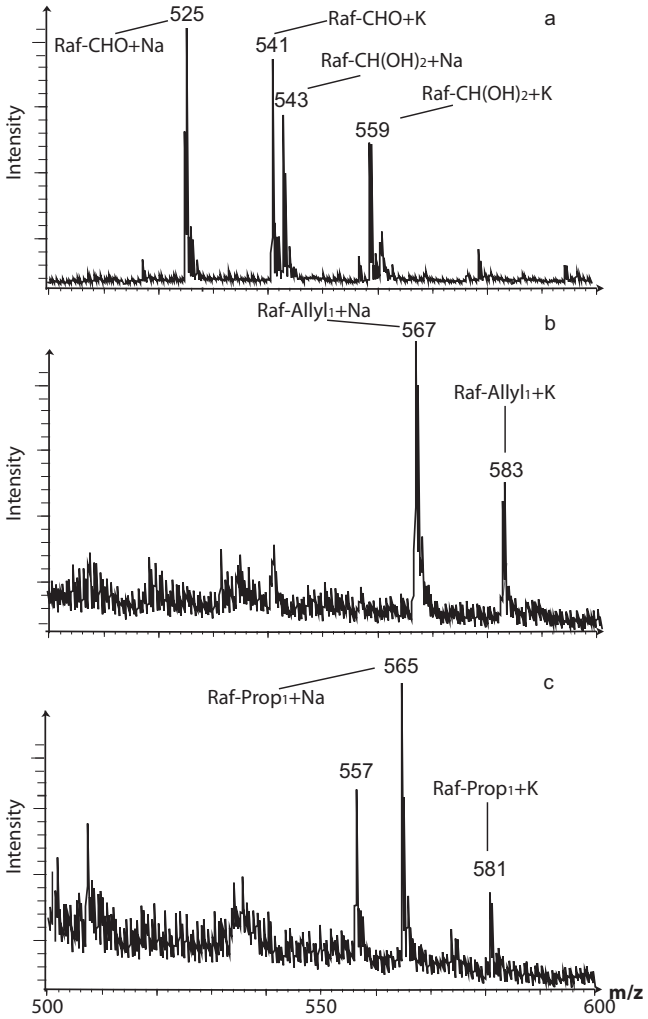


Fig. 2. MALDI-MS spectra of modified raffinose: (a) GO oxidized; (b) allylated (Allyl); and (c) propargylated (Prop) in Na^+ or K^+ adduct form. Raf-CHO = raffinose containing an aldehyde group.

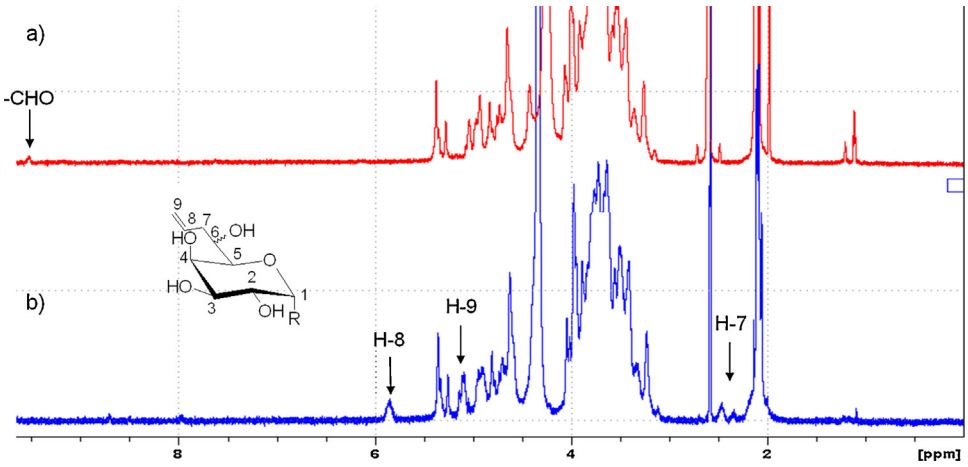


Fig. 3. ^{1}H NMR spectra (in DMSO-d_6/D_2O) of (a) oxidized GGM and (b) allylated GGM. R = GGM backbone.

earlier for the monosaccharide model compound, oxidized methyl galactopyranoside, using the same procedure, which also gave a yield of 100% (Leppänen et al., 2010).

Raf-CHO and cinnamyl chloride did react at room temperature; however, the reaction was not complete, since unreacted starting material could still be detected after 48 h. The conversion to product did not increase when raising the temperature to 55 °C.

Even if crotyl chloride reacts with oxidized methyl galactopyranoside to produce the corresponding homoallyl alcohol in moderate yield, no reaction could be observed with Raf-CHO. To enhance the solubility of crotyl chloride in the reaction media, the use of methanol as a co-solvent was attempted. Methanol did not improve the reaction and neither was it improved by performing the reaction at 55 °C.

Propargyl bromide and Raf-CHO did not react at room temperature, but at 55 °C the reaction proceeded smoothly and almost no starting material was detected after 24 h (Table 1). Even if the reaction proceeded well in just plain water, the rate could slightly be increased by using a co-solvent (MeOH or THF). MALDI–MS and ESI–MS analysis showed, in addition to trace amounts of the starting material and its hydrate form, only the expected product peak (m/z = 565, Na^+ adduct of propargylated raffinose) (Fig. 2 and Fig. S1 in supplementary data). Thus it was concluded that, as in the case of allyl bromide, no unwanted side-reactions occurred during the reaction between Raf-CHO and propargyl bromide.

3.2.2. Allylation and propargylation of oxidized GGM

Allylation of oxidized GGM at room temperature in plain water was not successful. However, when increasing the temperature to 55 °C, the reaction with allyl bromide was complete after 24 h. Already after 3 h, the DO had dropped to 20% (i.e. ~80% of the aldehydes had reacted), and after 24 h only trace amounts of aldehydes were left in GGM (Table 2). Allylation was verified by NMR by comparing ^{1}H NMR spectra obtained from the starting material and the product. The aldehyde proton signal at approx. 9.5 ppm had disappeared, and signals at approx. 2.3 ppm, 5.1 ppm and 5.8 ppm, corresponding to H-7, H-9, and H-8 respectively of allylated galactose, had appeared (Fig. 3). The formation of homoallyl alcohols was also verified by MALDI–MS analysis after enzymatic digestion of the modified polysaccharides (Fig. 4 and Table S1 in supplementary data). The introduction of an allyl group could be observed as an increase in mass by 40 molar mass units (m/z = 725.5, 771.6, 813.6, 891.7, 933.7, 1017.8, 1095.7, 1137.8), cinnamyl as 116 mass units (m/z = 805.7, 847.5, 889.5, 1009.6, 1051.7, 1171.7), and propargyl as 38 mass units (m/z = 727.3, 769.4, 811.5, 931.6, 973.6, 1093.6, 1135.6). In the case of GGM, the mass difference of 40 mass units could theoretically also indicate the occurrence of oligomers containing both oxidized galactose units and an acetyl group. But since the DO was 0% after allylation of oxidized GGM, we could safely assume that such oligomers were not present and the mass difference of 40 mass units could thus be assigned to the introduced allyl group.

As has been shown for the allylation of enzymatically oxidized methyl galactopyranoside, the addition of HCl (0.1 M) increases the rate of the reaction significantly (Leppänen et al., 2010). The use of 0.1 M HCl did also improve the rate of allylation of oxidized GGM; some reaction could be observed already at room temperature. After 24 h the DO had dropped to 49%, corresponding to a conversion of 38% of the available aldehyde groups. A decrease in pH has been shown to increase the dehydration of the aldehyde (Leppänen et al., 2010). Thus, even if HCl increases the rate somewhat, the final yield might decrease because of the dehydration. Although part of the decrease of DO in the reaction of oxidized GGM and allyl bromide in 0.1 M HCl can be assigned to the dehydration of aldehydes, the existence of the desired allylated galactose units could be verified by MALDI–MS and GC–MS analysis.

Both cinnamyl chloride and propargyl bromide reacted with oxidized GGM at 55 °C (Fig. 4 and Fig. S2 in supplementary data). The reaction with propargyl bromide was slightly more effective than the one with cinnamyl chloride. In the case of propargylation, 75% of the aldehydes had reacted, and for cinnamylation about 56% had reacted after 24 h (Table 2).

Metal mediated propargylation of carbonyl compounds theoretically produces mixtures of homopropargyl and allenyl alcohols. Because of the low degree of substitution (DS), the regioselectivity of indium mediated propargylation reaction of oxidized GGM could not be unambiguously assigned by NMR analysis. However, indium mediated propargylation of non-carbohydrate aldehydes in water has been reported to produce mainly the propargylic product (Isaac & Chan, 1995) and zinc mediated propargylation of carbohydrate aldehydes produce the homopropargyl alcohol as major products,

Table 2
Conversion in Barbier reaction of oxidized GGM and GM with different allyl halides.

Oxidized sugar	Halide[a]	DO(1)	DO(2)	Conversion [%][b]
GGM	Allyl	60	0	100 (6)
GGM	Prop	60	27	75 (5)
GGM	Cinn	60	45	45 (3)
GM_{high}	Allyl	84	70	56 (19)
GM_{low}	Allyl	40	27	45 (6)

DO(1) = DO of the starting material; DO(2) = DO of the product.
All reactions were carried out in water at 55 °C.
[a] Allyl = allyl bromide; Prop = propargyl bromide; Cinn = cinnamyl chloride.
[b] % of Gal-CHO that has reacted (% of total sugars).

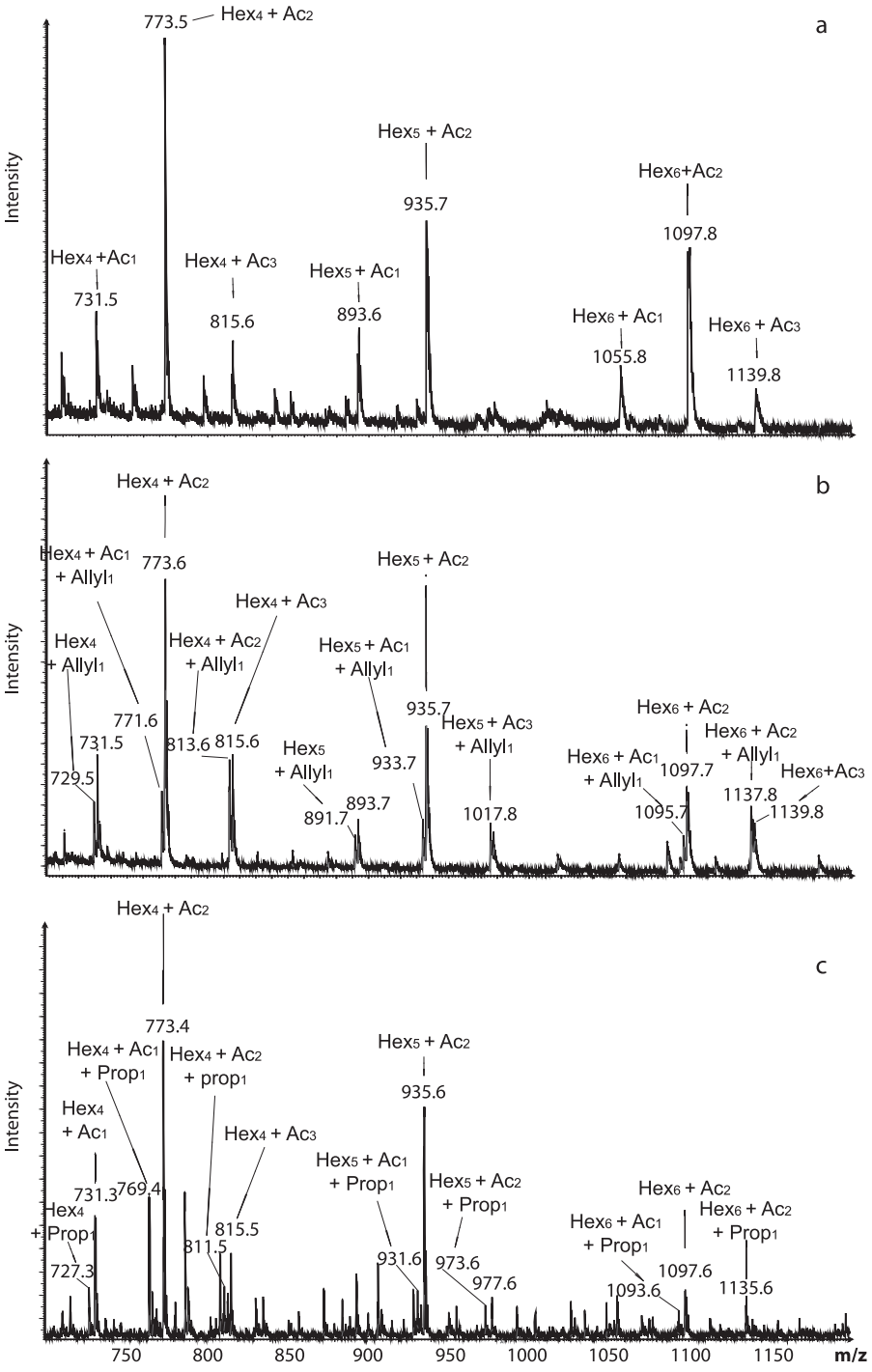


Fig. 4. MALDI–MS spectra of modified GGM's. GGM oligosaccharides produced by mannanase hydrolysis of (a) native GGM, (b) allylated GGM, (c) propargylated GGM in Na^+ adduct form. Hex = anhydrohexose; Ac = acetyl.

and only very little of the allenylic alcohols could been detected (Pakulski & Zamojski, 1997; Paulsen & Madsen, 2002).

3.2.3. Allylation and propargylation of GM and XG

To extend the usability of the indium mediated reaction also to other galactose-containing polysaccharides, oxidized GM and XG were allylated as well. All reactions were done in plain water and required 55 °C to work (Table 1). Oxidized XG reacted readily with allyl bromide, similarly to oxidized raffinose and GGM. Following EGase digestion, mono-allylated derivatives of the $Glc_4Xyl_3Gal_1$ XGOs (m/z = 1287.6, [M+Na^+]; m/z = 1303.7, [M+K^+]) and only di-allylated derivatives of the $Glc_4Xyl_3Gal_2$ XGO (m/z = 1489.8, [M+Na^+]; m/z = 1505.8, [M+K^+]) were observed in the MALDI analysis. Unreacted $Glc_4Xyl_3Gal_1$ XGOs (m/z = 1245.8, [M+Na^+]) and $Glc_4Xyl_3Gal_2$ XGO (m/z = 1405.9, [M+Na^+]) disappeared, while, the non-galactose Glc4Xyl3 XGO (m/z = 1085.7, [M+Na^+]) remained as a reference. The reaction was complete within 24 h and almost no starting material was left in the solution (Fig. 5). The ratio between galactose (Gal) and xylose (Xyl) in oxidized and allylated XG was determined, after reduction with $NaBD_4$ and methanolysis, by GC. A reduction in this ratio during reactions was assumed to occur because of allylation of the galactose units. The Gal:Xyl ratio of

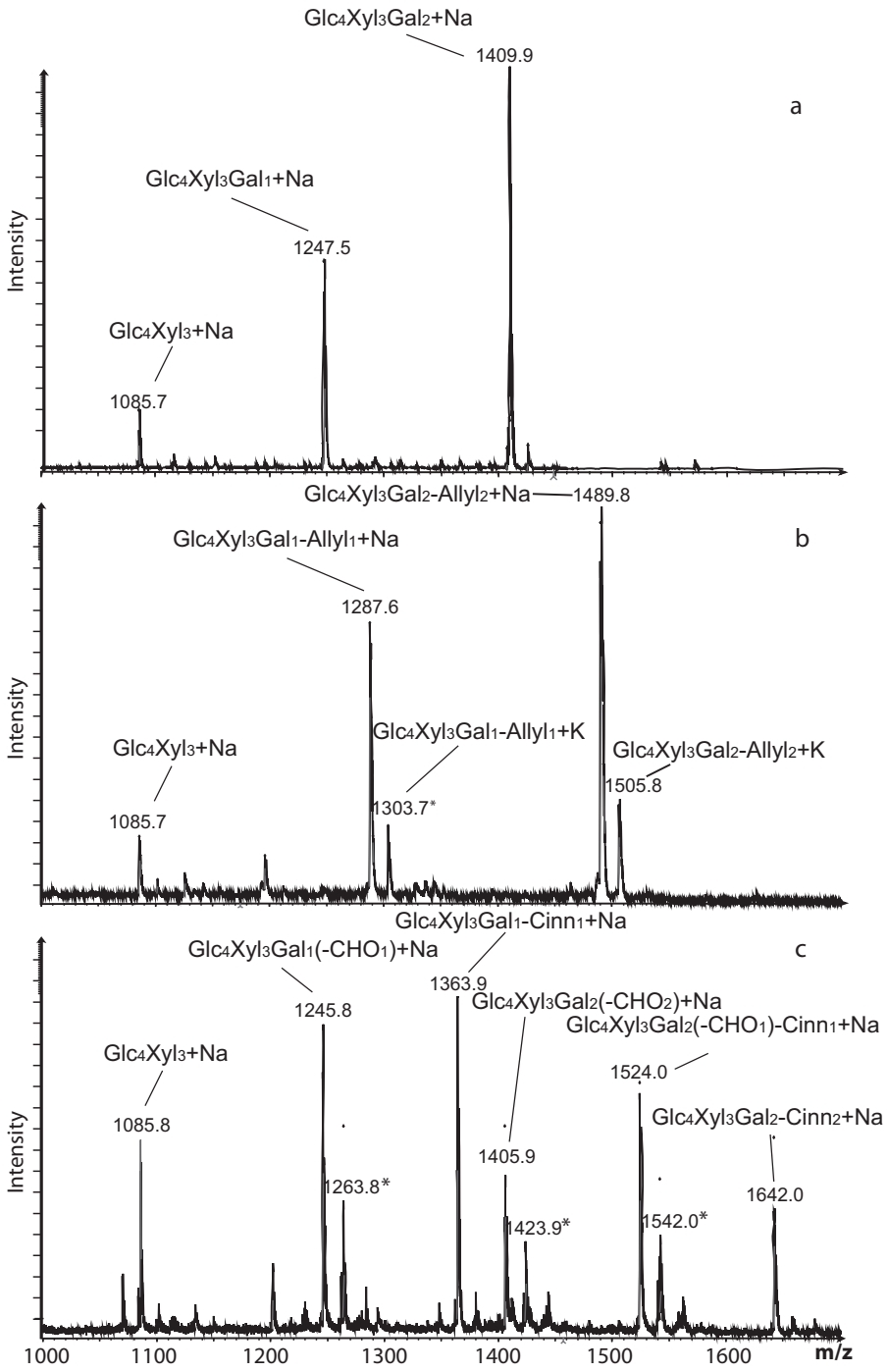


Fig. 5. MALDI–MS spectra of XG derivatives. XG oligosaccharides produced by EGase hydrolysis of (a) native XG, (b) allylated XG, and (c) cinnamylated XG in Na^+ or K^+ adduct form. Glc = glucose; Xyl = xylose; and Gal = galactose.

oxidized XG was 0.44. After allylation with allyl bromide, the ratio decreased to 0.06.

Enzymatically oxidized GM formed a thick gel, so the allylation reactions had to be performed in much more dilute solutions than allylation of the other polysaccharides. The lower concentration of aldehydes in the reaction solution might affect the outcome of the allylation; the reactivity of the aldehydes seemed to be remarkably lower than that of GGM or XG. The reaction was carried out on the oxidized GM's with DO's of 84% (GM_{high}) and 40% (GM_{low}). In both cases the result was similar, and around 50% of the aldehydes had reacted. Still, because of the high galactose content in GM, GM_{high} produced the polysaccharide containing the largest amount of allyl groups (Fig. S3 in supplementary data).

Oxidized XG reacted with cinnamyl chloride, but no reaction could be observed between XG and propargyl bromide (Table 1). The reaction between oxidized XG and cinnamyl chloride led to a Gal:Xyl ratio of 0.24, corresponding to decrease of approx 45% of the amount of galactose units in the starting material. The reaction was verified by NMR analysis (Figs. S4 and S5 in supplementary data). Quantification by comparing the anomeric signals to the aromatic ones in ^{13}C NMR gave a DS of 0.03. Following EGase digestion, mono-cinnamylated derivatives of the $Glc_4Xyl_3Gal_1$

XGOs (m/z = 1363.9, [M+Na$^+$]), and both the mono- (m/z = 1524.0, [M+Na$^+$]) and di-cinnamylated derivatives of the $Glc_4Xyl_3Gal_2$ XGO (m/z = 1642.0, [M+Na]) were observed (Fig. 5).

The amount of galactose side groups affects the reactivity of the polysaccharide. GM, containing the largest amount of galactose, exhibits the lowest reactivity. The aldehyde and hydroxyl groups form hemiacetal, and in the case of oxidized GM this leads to the formation of a thick gel. The allyl halides, barely soluble in water, are prevented from penetrating into the polysaccharide network and are sterically hindered from reaching the free aldehydes. Only allyl bromide reacted with oxidized GM, whereas the other, even less soluble allyl halides showed no reactivity at all.

4. Conclusions

Allyl functionalities can successfully be introduced to different galactose-containing polysaccharides, such as spruce galactoglucomannan, guar galactomannan, and tamarind xyloglucan. By combining oxidation by galactose oxidase with an indium mediated allylation reaction the galactose units can selectively be derivatized by allyl halides. The same reaction procedure can also be applied for spruce galactoglucomannan when using propargyl bromide as halide. All reactions were carried out by using water as the only solvent, thus the polysaccharides were functionalized in a one-pot reaction. Reactivity differences among the polysaccharides can be observed: oxidized GGM and XG reacted completely with allyl bromide, whereas for GM approx. only 50% of the aldehydes were allylated. Still, because of the high galactose content of GM, allylation of GM produces the polysaccharide with the highest DS.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2012.11.053.

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