

Amphiphilic Spruce Galactoglucomannan Derivatives Based on Naturally-Occurring Fatty Acids

Daniel Dax,^a Patrik Eklund,^b Jarl Hemming,^a Jawad Sarfraz,^c Peter Backman,^d Chunlin Xu,^{a,e,*} and Stefan Willför^a

A class of nonionic polysaccharides-based surfactants were synthesised from *O*-acetyl galactoglucomannan (GGM), water-soluble hemicelluloses from spruce, using naturally-occurring saturated fatty acids, $\text{CH}_3(\text{CH}_2)_n\text{COOH}$ ($n = 7, 12, \text{ and } 16$). Hemicelluloses can be recovered from waste-streams of papermaking and agricultural processes or isolated by hot water extraction of plant tissues integrated into a biorefinery process. Fatty acids can be recovered as byproducts of the agricultural and food industries. Different synthesis routes were applied to yield amphiphilic derivatives with either a grafted or block structure. Fatty acids activated with 1,1'-carbonyldiimidazole (CDI) were grafted to the backbone of GGM molecules on their hydroxyl groups. Alternatively, synthesised amino-activated fatty acids using ethylenediamine were reacted with the reducing end of GGM. By adjusting the reagent ratios, GGM-based surfactants with different hydrophilic to hydrophobic ratios were prepared. Their surface activity was assessed by measuring the surface tension in water. This study presents an approach to design carbohydrate-based surfactants using naturally-occurring fatty acids that may find potential applications in such areas as food, cosmetic, and paint formulations.

Keywords: Amphiphilic; Fatty acid; Hemicelluloses; Galactoglucomannan; Reductive amination; Surfactant; Surface tension

Contact information: a: Process Chemistry Centre, c/o Laboratory of Wood and Paper Chemistry, Åbo Akademi University, Porthansgatan 3, 20500 Åbo/Turku, Finland; b: Laboratory of Organic Chemistry, Åbo Akademi University, Biskopsgatan 8, 20500 Åbo/Turku, Finland; c: Laboratory of Physical Chemistry, Åbo Akademi University, Porthansgatan 3, 20500 Åbo/Turku, Finland; d: Laboratory of Inorganic Chemistry, Åbo Akademi University, Biskopsgatan 8, 20500 Åbo/Turku, Finland; e: Wallenberg Wood Science Center, KTH, 10044 Stockholm, Sweden; *Corresponding author: cxu@abo.fi

INTRODUCTION

In times of expanding energy needs, fossil oil and gas deposits are continuously exploited. However, due to the depletion of these resources, the interest in finding alternative sources for the production of energy, as well as petroleum-based chemicals and materials, is rising rapidly. Natural raw materials derived from plants and animals represent a very promising and environmentally friendly alternative to oil-based products. The large number of polysaccharides with a broad structural diversity and a versatile potential for chemical modifications constitute a highly interesting source for the production of novel and nontoxic materials. Polysaccharides are the most abundant natural material and can be obtained worldwide from various natural sources such as plants (Xu *et al.* 2008), seaweed (Lahaye and Robic 2007), bacteria (Carbonero *et al.* 2005), and animals (Taghizadeh and Davari 2006). In wood, hemicelluloses are the second most abundant polysaccharides after cellulose and are rapidly gaining importance

for several reasons. Hemicellulose has attracted great interest not only because its extraction and purification can be performed on a large scale (Willför *et al.* 2003a), but also because wood-based polysaccharides do not compete with food production in contrast to, *e.g.*, starch, which is a crucial point when using polysaccharides on an industrial scale. One of the most promising hemicelluloses is *O*-acetyl galactoglucomannan (GGM) from Norway spruce (*Picea abies*). GGM can be extracted from the wood matrix by pressurised hot water extraction (PHWE) (Al Manasrah *et al.* 2012) or can be isolated from thermomechanical pulp wastewaters by ultrafiltration (Willför *et al.* 2003b; Xu *et al.* 2009). GGM consists of a linear backbone of randomly distributed (1→4)-linked β-D-mannopyranosyl (Manp) and (1→4)-linked β-D-glucopyranosyl (GlcP) units, with α-D-galactopyranosyl (Galp) units as single side units (Hannuksela and Penhoat 2004). GGM is partially acetylated, and the *O*-acetyl groups are located randomly at the C2 and C3 positions of the mannose units in the main chain.

The desire to use natural polymers in different applications such as barrier films for food packaging (Peterson *et al.* 1999), hydrogels for controlled drug release (Hennink and van Nostrum 2002), or as additives in food (Moure *et al.* 2006), cosmetics, or paints has led to a wide range of modifications of polysaccharides. Therefore, either cellulose or non-cellulosic polysaccharides such as GGM are used. Several modifications of GGM have been reported using different synthetic approaches targeting the hydroxyl groups or the reducing end aldehyde group (Hartman *et al.* 2006; Kisonen *et al.* 2012, Halila *et al.* 2008). For chemical modifications of polysaccharides, in general, the hydroxyl groups of the sugar units can either react in a selective way, *e.g.*, only at the C6 position of the sugar units (Leppänen *et al.* 2010), or in a random reaction where all the hydroxyl groups can react (Voepel *et al.* 2011). To modify the hydroxyl groups of GGM, an activating agent is needed. A possible approach is to activate a carboxylic acid of a reactant with thionyl chloride, toluene-4-sulfonyl chloride, or 1,1'-carbonyldiimidazole (CDI) (Grote and Heinze 2005), whereby CDI allows gentle reaction conditions and is applied, *e.g.*, for cellulose esterifications (Hussain *et al.* 2004). The chemical nature of the reducing end sugar unit of a polysaccharide with its aldehyde form allows selective product formation by applying different reactions, such as reductive amination using an amino-functional reagent (Grefte *et al.* 2005; Zhang and Marchant 1994; Halila *et al.* 2008; Bernard *et al.* 2008, Lindqvist *et al.* 2013). In the case of a hydrophobic substituent being coupled to GGM, either at the hydroxyl groups along the polysaccharide chain or at the reducing end, amphiphilic derivatives are obtained. Similar derivatives were shown to have the ability to lower the surface tension (Garofalakis *et al.* 2000; Ferrer *et al.* 2002), stabilise emulsions (Zhang and Marchant 1996), or form microstructures in solutions (Houga *et al.* 2007). Although the goal of polysaccharide use is the development of biorenewable and biodegradable products, most of the modifications of GGM include the utilisation of petroleum-based components in the final products. Fatty acids occur in large amounts in plants and other living organisms and are widely used in industry for various applications. Fatty acids can be recovered as byproducts of the food industry (Cho *et al.* 2013). This, together with their ease of extraction, makes fatty acids a valuable starting material for designing new bio-based products (Grote and Heinze 2005).

For this study, the main objective was to use GGM and different naturally-occurring saturated fatty acids for the synthesis of amphiphilic GGM derivatives. Pelargonic acid (9:0), myristic acid (14:0), and stearic acid (18:0) were activated with CDI and subsequently reacted with the hydroxyl groups of GGM to yield GGM grafted with fatty acids (different degrees of substitution were reached in the final products). In a

second reaction path, the fatty acids were modified using ethylenediamine to result in amino-functional fatty acids. These amino-functionalised fatty acids were reacted with the reducing end of GGM in a reductive amination to form GGM block-structured derivatives. The amphiphilic products were analysed with nuclear magnetic resonance spectroscopy (NMR), Fourier transform infrared spectroscopy (FTIR), and high-pressure size exclusion chromatography – multi-angle laser light scattering and refractive index (HPSEC-MALLS/RI). Finally, the surface activity of the amphiphilic GGM derivatives was assessed *via* the surface tension in water.

EXPERIMENTAL

Materials

All materials used in the study are available commercially and were reagent grade or better and used without further purification. Di-*tert*-butyl-dicarbonate, ethylenediamine (EDA) $\geq 99.5\%$, sodium cyanoborohydride 95%, 1,1'-carbonyldiimidazole (CDI) 97%, trifluoroacetic acid (TFA) 99%, pelargonic acid $\geq 97\%$, myristic acid 99.5%, acetic acid anhydride (Riedel-de Haën), and methyl *tert*-butyl ether (MTBE) were purchased from SigmaAldrich. Stearic acid 99% and dimethyl sulfoxide (DMSO) were purchased from Merck. Ethanol ≥ 99.5 vol.-% was purchased from Altia Oyj. 1,4-Dioxane and pyridine were purchased from VWR, and acetone and tetrahydrofuran (THF) were purchased from J. T. Baker.

O-acetyl GGM from Norway spruce (*Picea abies*) was provided by The Finnish Forest Research Institute Metla after pressurised hot-water extraction. GGM was obtained as a solution with a concentration of 30 wt%. The extract was further purified to remove impurities and to narrow down the molar mass distribution (low polydispersity index (PDI)). Therefore, the concentrated GGM solution (330 mL) was diluted with water (670 mL) and then precipitated in 9 L of ethanol at room temperature. The colorless precipitate was filtrated off and washed with ethanol, acetone, and MTBE consecutively; finally, the solid GGM was freeze-dried. The purified GGM had a weight average molar mass (M_w) of 7.1×10^3 g/mol and a number average molar mass (M_n) of 4.7×10^3 g/mol (PDI ~ 1.5), as determined by HPSEC-RI/MALLS. The sugar ratio of GGM was determined by methanolysis to be 4~5:1:0.5~1.1 (Man:Glc:Gal) (Willför *et al.* 2008), and the degree of acetylation was (DS_{Ac}) ~ 0.20 .

Activation of the Carboxylic Acid of Stearic Acid

Stearic acid (5.0 g, 17.58 mmol) was dissolved in 50 mL of THF, and CDI (3.4 g, 21.1 mmol, 1.2 equiv) was added. The reaction mixture was stirred at room temperature, and the progression of the reaction was followed by observing the emission of CO₂. After the CO₂ formation ended, the reaction was stirred at room temperature for one additional hour. Subsequently, 100 mL of distilled water was added, and the precipitated product was filtered off, followed by freeze-drying from water to yield the imidazole derivative of stearic acid as a colourless powder (5.2 g, 15.6 mmol, 88% yield). ¹H NMR (600 MHz, CDCl₃, 35 °C): δ 0.89 ppm (tr, 3 H, -CH₂-CH₃), 1.22 to 1.45 (m, 28 H, CH₃-((CH₂)₁₄)-CH₂-), 1.81 (quint, 2 H, -CO-CH₂-CH₂-), 2.86 (tr, 2 H, -CO-CH₂-), 7.12 (s, 1 H, -CO-N-CH-), 7.50 (s, 1 H, -CO-N-CH-CH-), 8.17 (s, 1 H, -N-CH-N-).

Preparation of *tert*-Butyl-N-(2-aminoethyl) Carbonate

A solution of di-*tert*-butyl-dicarbonate (17.5 g, 80 mmol) in 180 mL of 1,4-dioxane was dropped into a stirred solution of ethylenediamine (36 g, 600 mmol, 7.5 equiv) in 180 mL of 1,4-dioxane over a period of 5 h at room temperature. After 48 h of stirring at room temperature, the formed precipitate was filtered off and the solvent was evaporated at a reduced pressure. Then, 300 mL of distilled water was added to the residue, and the water-insoluble bis(N,N'-*tert*-butyloxycarbonyl)-1,2-diaminoethane was removed by filtration (Sadhu *et al.* 2004). The product was extracted by dichloromethane from the aqueous solution saturated with NaCl. The organic phase was dried over Na₂SO₄, and the solvent was evaporated in vacuum to give *tert*-butyl-N-(2-aminoethyl) carbonate (NH₂-BOC) (7.68 g, 48 mmol, 60% yield). Proton nuclear magnetic resonance measurements were obtained as ¹H NMR (600 MHz, DMSO-*d*₆, 35 °C): δ 1.36 ppm (s, 9 H, (CH₃)₃-C-), 2.52 (tr, 2 H, -CH₂-NH₂), 2.90 (tr, 2 H, -CH-NH-).

Amidation of the Activated Stearic Acid with NH₂-BOC

The activated stearic acid (1 g, 3 mmol) was dissolved in 25 mL of THF at 45 °C under stirring. NH₂-BOC (0.86 g, 5.4 mmol, 1.8 equiv) was added to the reaction flask, and to catalyse the reaction, imidazole (0.41 g, 6 mmol, 2 equiv) was added. The reaction mixture was kept at 45 °C under stirring overnight, and then 60 mL of distilled water was added. The precipitated product was filtered off and freeze-dried. The dried product was dissolved in 25 mL of dichloromethane, and CF₃COOH (5 mL, 29 mmol, 10 equiv) was added. The reaction was stirred for 3 h at 40 °C, and subsequently, the solvent and the excess CF₃COOH were evaporated under reduced pressure. The residue was dissolved in dichloromethane and washed with a 0.5 M NaOH solution and with distilled water saturated with NaCl consecutively. The organic phase was dried over Na₂SO₄, and the solvent was removed in vacuum. The product (C18-NH₂) was obtained as a colorless solid (0.55 g, 2.8 mmol, 56% yield). ¹H NMR (600 MHz, DMSO-*d*₆, 35 °C): δ 0.82 ppm (tr, 3 H, CH₃-CH₂-), 1.18 to 1.24 (m, 14 H, CH₃-(CH₂)₁₄-CH₂-), 1.50 (tr, 2 H, -CH₂-CH₂-CO-), 2.08 (tr, 2 H, -CH₂-CO-), 2.89 (tr, 2 H, NH₂-CH₂-), 3.34 (q, 2 H, -CH₂-NH-CO-), 7.92 (tr, 1 H, -CH₂-NH-CO-), 8.11 (s, 2 H, -CH₂-NH₂).

Esterification of GGM

GGM (1 g, 0.2 mmol) was added to 15 mL of a mixture of dimethyl sulfoxide and THF (ratio 2:1) and heated up to 50 °C. Under stirring, activated stearic acid (134 mg, 0.4 mmol, 2 equiv) and imidazole (54 mg, 0.8 mmol, 4 equiv) were added. The reaction mixture was stirred at 50 °C for 15 h and then precipitated in ethanol. The precipitate was filtered off and washed with ethanol and acetone consecutively. After drying in a vacuum oven at 40 °C, the crude product was re-dissolved in distilled water and dialysed against distilled water for 48 h to remove solvent residues. Not all the products were soluble in water, and the water-soluble and water-insoluble fractions were separated. After removing the water by freeze-drying, the product fractions were obtained as a brownish solid (detailed yields and compositions are listed in Table 1). ¹H NMR spectra were recorded in D₂O at 35 °C, and the assignment of the peaks (resp. peak regions) can be found in Fig. 2.

Reductive Amination of GGM

GGM (1 g, 0.2 mmol) was dissolved in 50 mL of a mixture of dimethyl sulfoxide and ethanol (ratio 3:2). C18-NH₂ (327.5 mg, 1.0 mmol, 5 equiv) was added to the

reaction flask, and the mixture was heated up to 50 °C. After stirring for 30 min, a homogenous solution was obtained and NaBH₃CN (628 mg, 10 mmol, 50 equiv) was added. After 48 h of stirring at 50 °C, a second portion of NaBH₃CN (628 mg, 10 mmol, 50 equiv) was added and the reaction was stirred for another 24 h and then stopped. The reaction mixture was precipitated in ethanol and recovered by centrifugation. The precipitate was washed with ethanol and acetone consecutively and then dried in the vacuum oven for 48 h at 40 °C. The product was re-dissolved in distilled water and dialysed against distilled water to remove dimethyl sulfoxide residues. After the dialysis, the water was removed by freeze-drying, and the product was obtained as a light brownish solid (420 mg, 0.078 mmol, yield 40%). ¹H NMR spectra were recorded in D₂O at 35 °C, and the assignment of the peaks (resp. peak regions) can be found in Fig. 3.

High-Pressure Size Exclusion Chromatography (HPSEC)

M_w and M_n of native GGM were determined using water as a solvent. The determined number-average molar mass ($M_n=4.7\times 10^3$ g/mol) was used for the calibration of the ¹H NMR spectra. The HPSEC measurements of the GGM-fatty acid derivatives, using water as the solvent, showed unsatisfactory results. Therefore, the synthesised products were acetylated and dissolved in THF for determination of their M_n and M_w values.

M_w and M_n , and the molar mass distribution (MWD) of native GGM were determined by HPSEC in an on-line combination with a MALLS instrument (miniDAWN, Wyatt Technology, Santa Barbara, USA) and a RI detector (Shimadzu Corporation, Japan). A two-column system, 2 x Ultrahydrogel™ linear 300 mm x 7.8 mm column (Waters, Milford, USA), was used in series. The elution solvent was 0.1 M NaNO₃. The flow rate was 0.5 mL/min. A dn/dc value of 0.150 mL/g was used (Michielsen *et al.* 1999). The samples were filtered through a 0.22- μ m nylon syringe filter before injection. The injection volume was 100 μ L. Astra software (Wyatt Technology, Santa Barbara, USA) was used to analyse the data.

A HPSEC system using polystyrene standards for calibration after recording retention volumes with a SEDEX Model 85 ELSD detector (SEDERE, Alfortville France) was also applied. A two-column system, 2 x JordiGel DVB 500Å 300 mm x 7.8 mm (Jordi Labs, Mansfield, MA, USA), was used in series. THF containing 1% (v/v) acetic acid was used as the elution solvent, and the flow rate was 0.8 mL/min. The data were analysed with Shimadzu software packages CLASS VP 6.1 and GPC for CLASS VP (Shimadzu Corporation, Japan). Samples were prepared as follows: native GGM and the GGM-fatty acid derivatives were acetylated to increase hydrophobicity. Therefore, 2 mg of material were treated with 250 μ L of pyridine and 250 μ L of acetic acid anhydride at 70 °C for 2 h. After removing the excess acetic acid anhydride and pyridine, the derivatised sample was redissolved in 2 mL of THF and filtered through a 0.45- μ m PTFE syringe filter.

Nuclear Magnetic Resonance Spectroscopy (NMR)

The native GGM and the GGM-fatty acid derivatives were analysed by ¹H and ¹³C NMR measurements using a Bruker Avance spectrometer (operation frequency: ¹H: 600.13 MHz; ¹³C: 150.92 MHz). For the water-soluble products, D₂O was used as a solvent, and the water-insoluble products were dissolved in DMSO-*d*₆. For the ¹³C measurements in water, DMSO-*d*₆ was used as an internal standard. The temperature for all the experiments was set to 35 °C.

Fourier Transformed Infrared Spectroscopy (FTIR)

The infrared spectroscopy measurements were performed with a Bruker ALPHA series using the ALPHA platinum ATR single-reflection diamond ATR module. The samples were directly placed on the ATR plate for measurement. The results were evaluated using the software OPUS from Bruker.

Thermal Gravimetric Analysis (TGA)

The thermal stability of GGM and GGM-fatty acid derivatives was investigated by DSC-TGA (differential scanning calorimetry-Thermal Gravimetric Analysis) (Q600, Ta Instruments). The samples were dried before thermal analysis. The heating rate in the experiments was 10 °C/min up to 600 °C under nitrogen. A platinum cup was used in these tests. The weights of the sample and the DTA-signals (the difference between the sample and reference temperature) were recorded.

Determination of the Surface Tension in Water

The surface tension of the GGM-fatty acid derivative solutions was measured using the Du Noüy ring method with a KSV Sigma 70 Tensiometer. The used Pt-Ir ring was rinsed with ethanol with consecutive drying after each measurement to ensure a zero contact angle. The real tension values were immediately determined for different concentrations of the surfactants at 25 °C. The equilibrium time for all the samples was set to 5 min. The surface tensions were plotted versus the logarithm of the surfactant concentration (mg/mL), and the respective critical aggregation concentrations (CAC) were determined graphically from the change of the slope.

Determination of the Area (A) Occupied by the Adsorbed Molecules at the Water-Air Interface

The surface excess, Γ (mol/m²), was calculated using the Gibbs adsorption isotherm as shown in equation (1),

$$\Gamma = - (1 / RT)(d\gamma / d \ln C) \quad (1)$$

where R is the ideal gas constant (8.314 J/(mol*K)) and T is the absolute temperature during the measurement (Jönsson *et al.* 1998). $d\gamma/d \ln C$ was estimated from the plots of the respective surface tension versus the natural logarithm of the concentration of the surfactant when the concentration approaches the CAC. In the cases where the CAC could not be determined because of precipitation of the sample at high concentrations, the slope was estimated using the data points available. The cross sectional area per surfactant molecule, A (Å²), was subsequently calculated from Equation (2),

$$A = 1 / (N_A \Gamma) \quad (2)$$

where N_A is Avogadro's number. The water used for the surfactant solutions was purified by a Millipore system and had a surface tension of ~73 mN/m.

Calibration of the ¹H NMR Spectra

For quantitative evaluation of the proton spectrum, the theoretical integration value for the peak region from 3.2 to 4.4 ppm was determined. Beforehand, size

exclusion chromatography for native GGM and a volumetric titration for the determination of the acetyl-group content were performed. For the calculations, the average molar mass ($M_n = 4.7 \times 10^3$ g/mol) was used. The volumetric titration gave a value of 1.16 mmol/g (acetyl group per gram of GGM). The amount of acetyl groups per GGM chain was calculated as shown in equation (3),

$$n_a = M_n \times 1.16/1000 = 4700 \times 1.16/1000 = 5.5 \quad (3)$$

where M_n is the number average molar mass of GGM and n_a is number of acetyl-groups per GGM chain (degree of acetylation).

The degree of acetylation of GGM was 5.5 (referred to one GGM chain). The amount of sugar units in a GGM chain with a molecular weight of 4.7×10^3 g/mol containing 5.5 acetyl groups per macromolecule was determined using equation (4), knowing that the average molecular weight of each anhydro-monomer unit was $M_s=162$ g/mol,

$$n_s = (M_n - n_a \times M_a)/M_s = (4700 - 5.5 \times 43)/162 = 27.6 \quad (4)$$

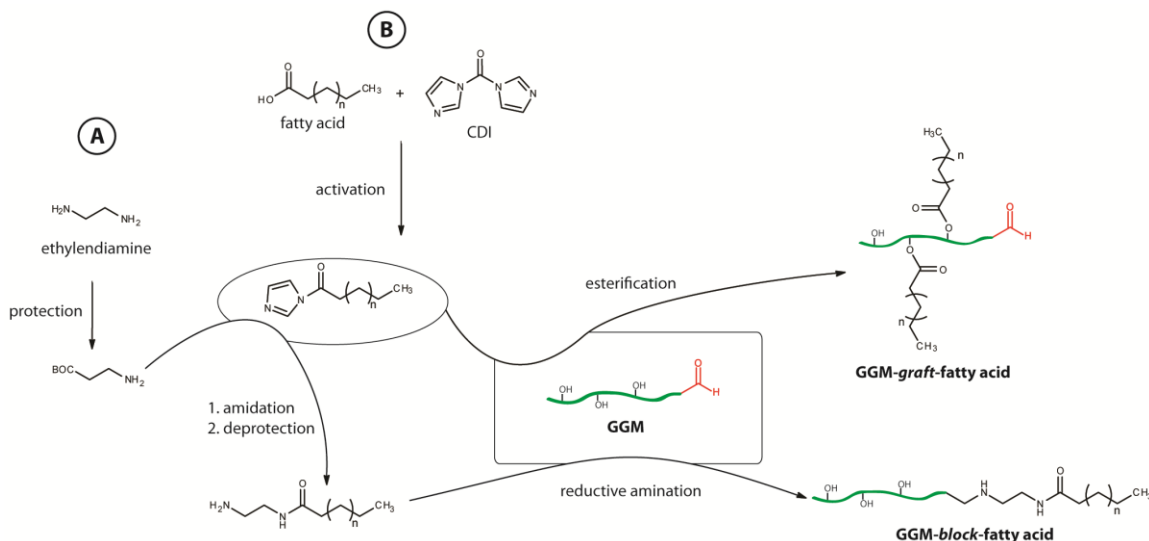
where M_n is the number average molar mass of GGM, n_a is the number of acetyl-groups per GGM chain, M_a is the molar mass of one acetyl-group, and M_s is the average molar mass of one sugar unit in GGM.

The total mass of the 5.5 acetyl groups was subtracted from M_n , and it was found that, on average, one GGM chain consisted of 28 sugar units. For the determination of the theoretical value for the integration of the area between 3.2 and 4.4 ppm in the proton NMR spectra, the amount of protons showing a signal in this area had to be determined. Every sugar unit has 7 protons, which can be detected by proton NMR. With an average of around 28 sugar units per chain, there were a total of 196 protons for each GGM chain. There are two distinguished regions in the proton spectrum of GGM, the first from 3.2 to 4.4 ppm and the other from 4.6 to 5.5 ppm. The region between 4.6 and 5.5 ppm contains signals of the reducing end protons as well as the protons in the neighbourhood of the acetyl groups, resulting in $28+5.5=33.5$ protons. Subtracting 33.5 protons from the total number of protons gave 162.5 protons, which was taken as the theoretical value for the integration of the peak region from 3.2 to 4.4 ppm. This value was set as a constant for all the spectra evaluated in this work, and the products were determined to be solvent-free before measuring the ^1H NMR spectra.

RESULTS AND DISCUSSION

In the present paper, a method to synthesise grafted and block-structured derivatives of GGM using different naturally occurring fatty acids is presented. The grafting reactions were performed through random esterification of the hydroxyl groups of GGM using 1,1'-carbonyldiimidazole (CDI) as an activating agent for the fatty acids. For the synthesis of the block-structured products, amino-functional fatty acids were coupled to GGM by reductive amination (Scheme 1). The different derivatives were characterised by NMR and SEC and preliminarily tested in terms of their ability to lower the surface tension of water. To simplify the nomenclature of the synthesised

intermediate and final products, the fatty acids used are labelled by the amount of carbon atoms in the molecule (pelargonic acid: C9; myristic acid: C14; stearic acid: C18).



Scheme 1. Reaction equation for the formation of GGM block (A) and grafted fatty acid (B) derivatives

Modification of Fatty Acids

The activation of the fatty acids was performed using CDI, which was chosen as an activation reagent because mild reaction conditions could be applied and the resulting products could easily be purified. Three different fatty acids were activated this way: pelargonic acid (C9-imidazole), myristic acid (C14-imidazole), and stearic acid (C18-imidazole). The products were analysed by ^1H NMR spectroscopy. By comparing the signals of the terminal CH_3 -group of the fatty acids at 0.88 ppm ($-\text{CH}_2-\text{CH}_3$) and the signals of the imidazole ring at 7.11, 7.50, and 8.18 ppm, the conversions could be calculated and were around 90% for all the fatty acids.

The activated fatty acids were suitable for the esterification of GGM, but they were also a precursor for the synthesis of amino-functional fatty acids. For generating amino-functional fatty acids, the fatty acids were reacted with *tert*-butyl-*N*-(2-aminoethyl) carbonate (DE-BOC) with consecutive deprotection of the amine group. Fatty acids with terminal amino groups (C9- NH_2 , C14- NH_2 , and C18- NH_2) were obtained with good yields (> 56%) and conversions around 95%. The conversion of the activated fatty acids was determined with ^1H NMR spectroscopy by comparing the signals of the terminal CH_3 -group of the fatty acids at 0.88 ppm ($-\text{CH}_2-\text{CH}_3$) and the CH_2 -group in the neighbourhood of the terminal amine at 2.89 ppm ($-\text{CH}_2-\text{NH}_2$).

Esterification of GGM

The esterification of GGM with fatty acids was optimised from a previously reported method for starch (Grote and Heinze 2005) by applying a lower temperature and using a different solvent mixture. The esterification of GGM using the different activated fatty acids (C9-imidazole, C14-imidazole, and C18-imidazole) was performed in a mixture of dimethyl sulfoxide and THF (ratio 2:1) in the presence of imidazole as a catalyst. For each activated fatty acid, three different molar ratios in respect to GGM were used (2, 4, and 8 equivalents). For several reactions, not all the products were

soluble in water and two product fractions were separated by filtration. In Table 1, the results of the esterification reactions are listed.

Table 1. Results of the Esterification and Reductive Amination Reactions

Product ^a	Molar ratio ^b GGM:fatty acid	Yield (%)	M _n (g/mol) ^b	M _w /M _n ^b	DS ^c
Water soluble					
GGM	-	-	6057	2.76	-
GGM- <i>g</i> -C9 (0.80)	1:2	34	6382	2.14	0.029
GGM- <i>g</i> -C9 (1.53)	1:4	25	6821	2.14	0.055
GGM- <i>g</i> -C9 (2.23)	1:8	30	7328	1.91	0.080
GGM- <i>g</i> -C14 (0.82)	1:2	31	6945	1.96	0.029
GGM- <i>g</i> -C14 (1.14)	1:4	16	6067	2.07	0.041
GGM- <i>g</i> -C14 (1.95)	1:8	15	7288	1.66	0.070
GGM- <i>g</i> -C18 (0.68)	1:2	35	6744	2.3	0.024
GGM- <i>g</i> -C18 (1.07)	1:4	30	7191	2.02	0.038
GGM- <i>g</i> -C18 (1.42)	1:8	34	8804	2.02	0.051
GGM- <i>b</i> -C9	1:5	45	6036	2.43	-
GGM- <i>b</i> -C14	1:5	57	6769	2.68	-
GGM- <i>b</i> -C18	1:5	35	6679	2.15	-
Water-insoluble					
GGM- <i>g</i> -C9 (4.85)	1:8	5	12775	1.79	0.173
GGM- <i>g</i> -C14 (2.51)	1:2	19	9084	2.11	0.090
GGM- <i>g</i> -C14 (3.13)	1:4	18	10372	1.95	0.112
GGM- <i>g</i> -C14 (5.45)	1:8	21	10978	1.95	0.195
GGM- <i>g</i> -C18 (1.68)	1:2	8	7769	2.2	0.060
GGM- <i>g</i> -C18 (3.42)	1:4	30	9436	1.85	0.122

^a In brackets are the average numbers of fatty acid per GGM chain in the final products; ^b Molar mass determination after acetylation of the GGM and the GGM derivatives (procedure in "HPSEC" in the experimental section); ^c Degree of substitution

In the ¹H NMR spectra, the area from 3.20 to 4.37 ppm was set to 162.5, which represents the protons in the GGM chain apart from the anomeric protons (see section "Calibration of the ¹H NMR spectra" for further information). The degree of substitution (DS) of the fatty acid chains was determined by integrating the signal of the terminal CH₃-group (-CH₂-CH₃) at 0.88 ppm. The incorporated fatty acids in the GGM chain increased for each of the activated fatty acids when the molar ratio of the fatty acid during the reaction was higher. For a low molar ratio of 1:2 (GGM: activated fatty acid) during the reaction, the water-soluble fractions contained parts of native GGM that had not reacted (fatty acid per GGM (FA/GGM) value < 1). The yields of the water-soluble and water-insoluble fractions were relatively low for all the reactions. This is assumed to be due to a loss during the precipitation, where parts of the products might be soluble in ethanol (especially for high DS values). For a molar ratio of 1:2 during the reaction, the DS for the water-soluble products was similar for all fatty acids. For higher molar ratios

(1:4 and 1:8), the highest DS of the water-soluble products was reached for C9-imidazole and decreased for C14-imidazole and C18-imidazole. All the esterifications of GGM with C14-imidazole and C18-imidazole resulted in a water-soluble and a water-insoluble fraction, whereas for C9-active, only the highest ratio (1:8) gave a small amount of water-insoluble products. Overall, GGM grafted products with three different fatty acids could be synthesised reaching DS values from 0.023 to 0.180. The higher the molecular weight of the fatty acid and the DS were, the less water-soluble were the resulting products. For all the collected products, no deacetylation of the GGM chain was observed.

Reductive Amination of O-acetyl Galactoglucomannan

The formation of defined block-structured products of GGM and various fatty acids was performed by reductive amination using the synthesised amino-functional fatty acids (C9-NH₂, C14-NH₂, and C18-NH₂). In contrast to the esterification reactions, the reductive amination reactions yielded only water-soluble products. The conversion of the reducing end of GGM was followed by a decrease in the intensity of the reducing end signals of GGM in the proton spectra (see “¹H NMR spectroscopy” for further information). At the same time, the intensity of the signals belonging to the respective fatty acid increased. The complete formation of the final product was verified by comparing the signal of the terminal CH₃-group (-CH₂-CH₃) at 0.88 ppm of the fatty acids to a calibrated peak region of GGM from 3.2 to 4.4 ppm (set to 162.5 protons). The yields of the block-structured products were comparable for the different fatty acids (see Table 1). The relatively high loss of the amphiphilic products is assumed to be due to the formation of ethanol-soluble structures (*e.g.*, micelles) during the precipitation step that could not be collected during filtration. As for the esterification reactions, no deacetylation of the GGM chain was observed.

FTIR Spectroscopy

FTIR spectra for the esterified and the reductively aminated products were recorded at room temperature. The band of valence vibration of the methylene groups of the fatty acids at 2850 and 2918 cm⁻¹ increased with increasing DS of the products (Fig. 1). Also, a slight increase in the carbonyl-group signal at 1731 cm⁻¹ (ν_sC=O) was observed.

¹H NMR Spectroscopy

Figure 2 shows the proton spectra of GGM and the esterified derivatives of GGM and pelargonic acid. The region from 4.6 to 5.5 ppm includes the anomeric protons (H-1 and H-1'') of the sugar units as well as the proton located close to an acetyl group (H-9) (Hannuksela and Penhoat 2004). The CH₃-group (H-7) of the acetyl groups gave signals in the region between 2.11 and 2.28 ppm. The peak at 0.88 ppm belongs to the protons of the terminal CH₃-group of the fatty acid chain (H-16), and the peak at 1.31 ppm belongs to the CH₂ groups (H-13, 14, 15) of the fatty acid. The CH₂ groups close to the carbonyl-function H-11 and H-12 gave separated signals at 2.49 and 1.66 ppm, respectively. Due to the complexity of the GGM spectrum, it was not possible to define which position (C2, C3, or C6) was preferred during the esterification reactions with the activated fatty acids. The integrals of the signals belonging to the fatty acids get stronger with a higher degree of substitution; this can be observed with a growth of the intensity of the peaks belonging to the fatty acids (Fig. 2).

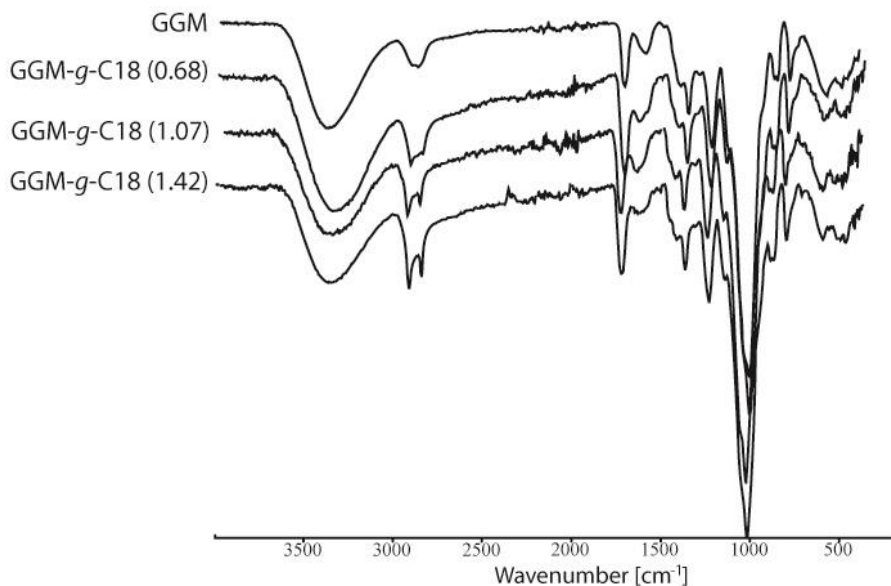


Fig. 1. FTIR spectra of native GGM and GGM grafted with stearic acid with different DS values

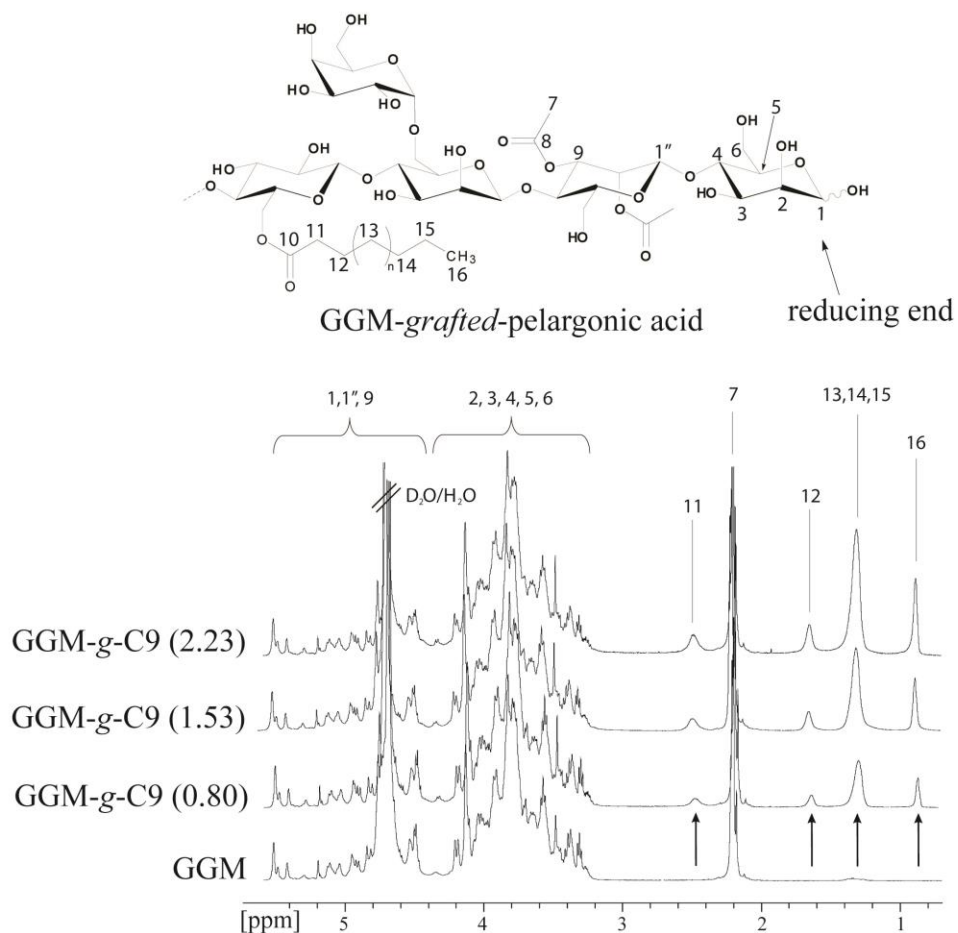


Fig. 2. ¹H NMR of native GGM and the esterification product of GGM and pelargonic acid in D₂O

The block-structured products resulting from the reductive amination of GGM with amino-functional fatty acids were also analysed by ^1H NMR spectroscopy. In Fig. 3, the spectra of GGM and the product of the reductive amination of GGM and amino-functional pelargonic acid (C9-NH_2) are shown. The peaks of GGM and the fatty acids were assigned the same way as described above for the esterification products. The protons of the CH_2 -groups (H-17 and H-18) of C9-NH_2 overlap the signals of GGM in the peak region between 3.2 and 4.4 ppm. The complete conversion of the reducing end was verified by the complete disappearance of the reducing end signals of GGM at 4.93 ($\alpha\text{-Manp}^{\text{R}}$) and 5.20 ppm ($\beta\text{-Manp}^{\text{R}}$) (Fig. 4). These two peaks were assumed to be the anomeric protons of the mannose end groups of GGM in comparison to the reducing end signals of manno- β -D-glucopyranose. Even though there are also glucose units situated at the reducing end of GGM (assigned in ^{13}C -NMR spectra), no signals could be isolated in the proton spectra.

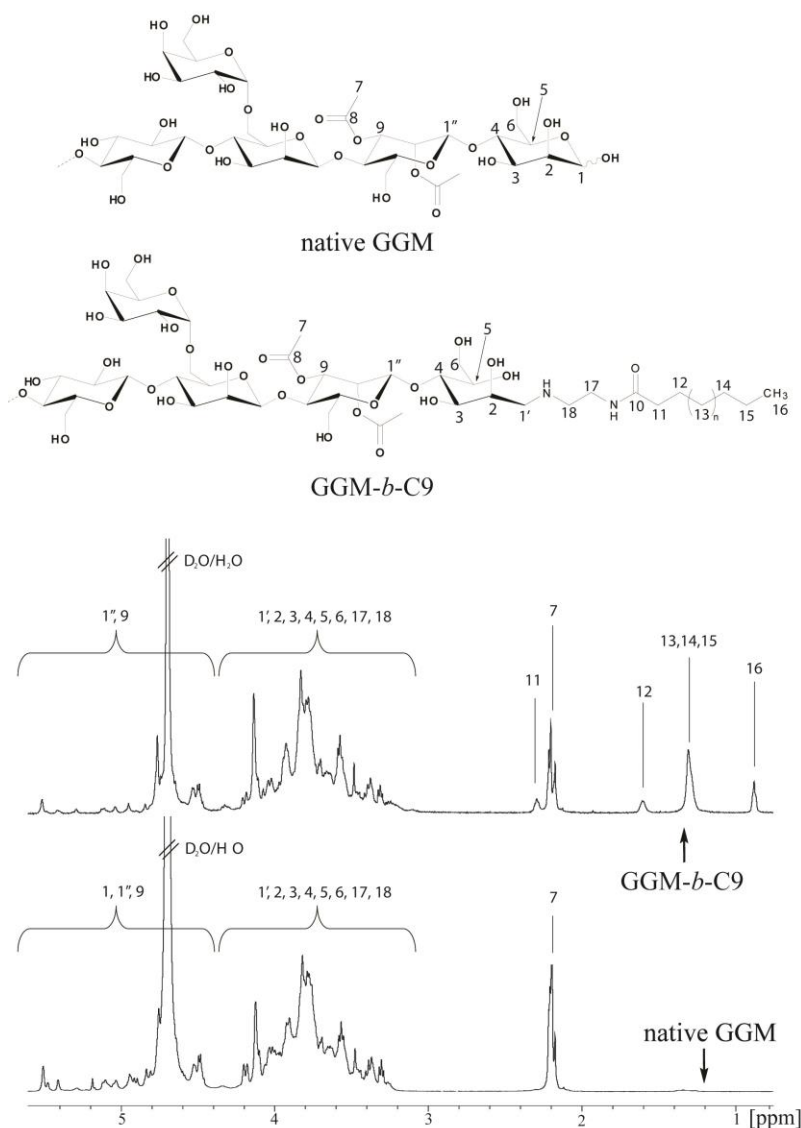


Fig. 3. ^1H NMR of native GGM and its block-structured derivative with amino-functional pelargonic acid in D_2O

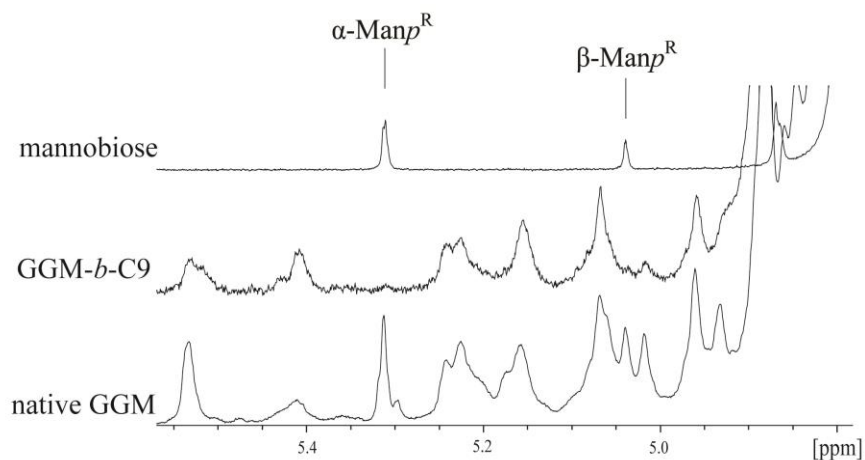


Fig. 4. Magnification of the reducing end peak region of GGM and the GGM block-structured derivative with amino-functional pelargonic acid. As a reference, the reducing end peaks of mannobiose are shown.

¹³C NMR Spectroscopy

For the products of the esterification reactions and reductive amination, the carbon signals of the fatty acid chains were detected in the peak region between 13.93 and 32.05 ppm (Fig. 5).

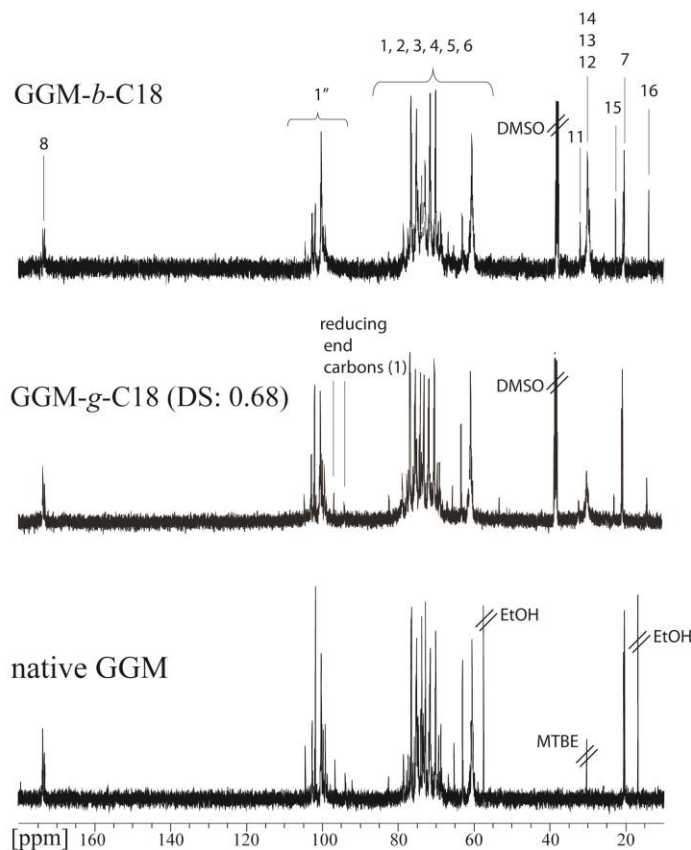


Fig. 5. ¹³C NMR spectra of GGM, GGM grafted with stearic acid, and the GGM block-structured derivative of stearic acid in D₂O

The CH₃ carbon (C-16) gave a signal at 14.11 ppm, and the CH₂ group (C-15) next to it gave a signal at 22.77 ppm. The carbons in the middle of the fatty acid (C-12, C-13, and C-14) gave signals in the peak region between 28.66 and 30.74 ppm. The carbon next to the carbonyl carbon (C-11) had a signal at 31.88 ppm. The carbonyl carbon of the fatty acid ester could not be detected and thus was not separately identified in the peak region from 172.89 to 173.73 ppm. The signals in the region from 92.11 to 104.51 ppm were assigned to the anomeric carbons of GGM (Fig. 6). The α -Manp^R and β -Manp^R have a chemical shift of 94.86 and 94.72 ppm, respectively, assigned in a previous work (Hannuksela and Penhoat 2004). To identify the peaks at 92.11 and 96.82 ppm, a spectrum of cellobiose was recorded. By comparing the spectrum of cellobiose and native GGM, the signal at 92.11 ppm was assigned to α -Glu^R, and the signal at 96.82 ppm was assigned to β -Glu^R. These four signals completely disappeared in the spectra of the reductive amination products, which is proof of the successful conversion of all the GGM reducing ends during the reaction.

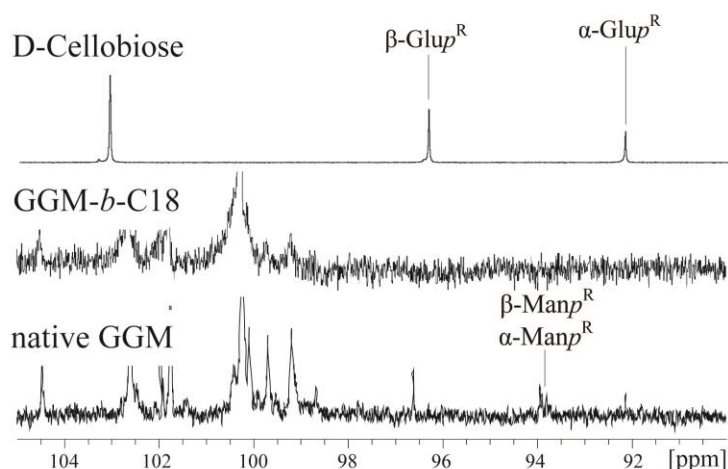


Fig. 6. Magnification of the reducing end peak region of native GGM and its block-structured fatty acid derivative. As a reference, the spectrum of cellobiose is shown.

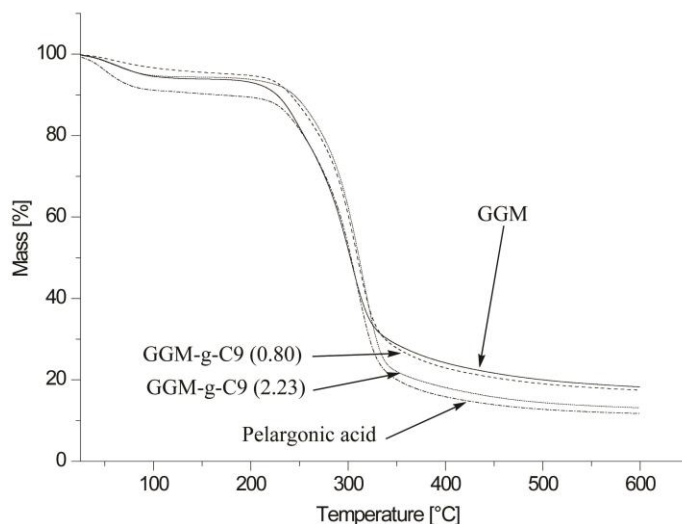


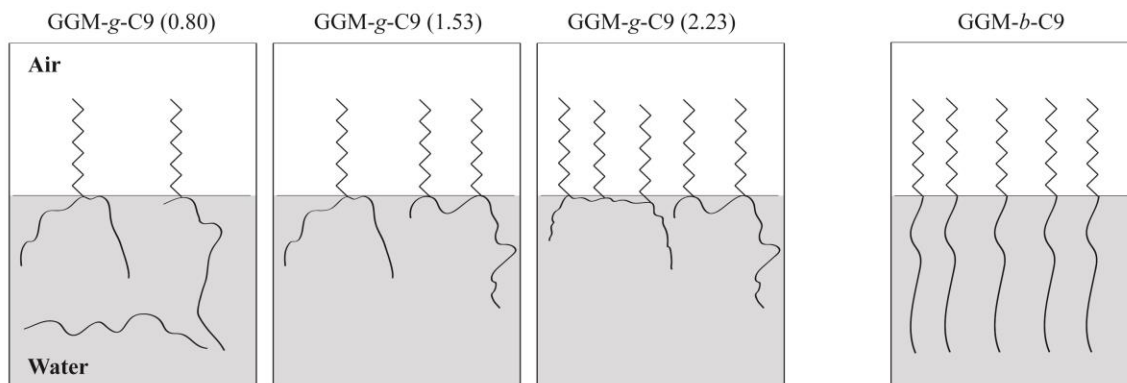
Fig. 7. TGA thermograms for GGM, pelargonic acid, and the GGM grafted derivatives of pelargonic acid

Thermal Stability

Thermal gravimetric analysis of GGM and its derivatives with various fatty acids was also conducted. Figure 7 displays the exemplary results of GGM-*g*-C9, as well as for native GGM and pelargonic acid (C9). The TGA of native GGM and the GGM-pelargonic acid derivatives shows a mass loss in two stages. The first stage between 21 and 165 °C corresponds to a mass loss of 4 to 7%. This mass loss is assumed to have its origin in the loss of water or solvent residues adsorbed or bound to the products. In a second stage between 210 and 600 °C, the mass loss was between 76 and 85%, with a dramatic loss between 210 and 350 °C. In comparison with native GGM, pelargonic acid is less thermally stable and shows larger mass losses (10% in the range between 21 and 165 °C and 86% in the range between 210 and 600 °C). Native GGM shows the highest thermal stability, and its esterification derivatives with pelargonic acid show a trend of being less thermally stable as the pelargonic acid content increases. The block-structured derivative of GGM and pelargonic acid did not follow this trend and showed a similar thermal stability to the esterification product with a pelargonic acid/GGM ratio of 2.23. The TGA results for the GGM-myristic and GGM-stearic acid derivatives showed the same trends (data not shown).

Surface Tension of Amphiphilic GGM Derivatives

Detailed studies of the GGM-fatty acid derivatives with respect to their surface activity in water were performed. For this, solutions with different concentrations of GGM-*g*-C9 (fatty acid/GGM: 0.80, 1.53, 2.23), GGM-*g*-C14 (0.82, 1.14, 1.95), and GGM-*g*-C18 (0.68, 1.07, 1.42), as well as the respective block-structured products of GGM and the fatty acids GGM-*b*-C9, GGM-*b*-C14, and GGM-*b*-C18, were prepared. The results of the surface tensiometric measurements are shown in Fig. 8. The critical aggregation concentration (CAC) values were determined by the break-points of the surface tension isotherms, which were estimated from the intersection of the fitted lines of the descending and flat part of the curves (Ferrer *et al.* 2002). For the products GGM-*g*-C14 (1.14), GGM-*g*-C18 (0.68), GGM-*g*-C18 (1.07), and GGM-*g*-C18 (1.42), the CAC could not be determined because the products were not soluble at high concentrations (no plateau could be observed in the plots). For those products for which the CAC was possible to determine, the minimum surface tension γ_{\min} was determined and ranged between 44 and 57 mN/m.



Scheme 2. Hypothetical assembling of the GGM grafted with pelargonic acid (left) and GGM block-structured derivative of pelargonic acid (right)

To understand the large differences between the minimum surface tensions, the surface excess Γ and the cross-sectional area per molecule A were calculated (Table 2). The grafted and the block-structured products needed to be evaluated separately. For GGM-*g*-C9 and GGM-*g*-C14, a higher degree of substitution led to a lower minimum surface tension and a simultaneous decrease of the cross-sectional area per molecule; however, the CAC is similar for the respective product series (same fatty acid). Considering one series of the fatty acid grafted GGM derivatives, the derivative with a higher degree of substitution was more surface active, presumably ascribed to more hydrophobic chains located at the surface when forming a closely packed monolayer (Scheme 2).

Table 2. Properties of Amphiphilic GGM Derivatives in Water

Surfactant	CAC (mg/mL)	γ_{\min} (mN/m)	Γ (mol/m ²)	A (Å ²)
GGM	-	68.8	-	-
GGM- <i>g</i> -C9 (0.80)	1.28	55.0	1.5	111
GGM- <i>g</i> -C9 (1.53)	1.00	50.0	1.6	102
GGM- <i>g</i> -C9 (2.23)	1.22	44.4	1.7	99
GGM- <i>g</i> -C14 (0.82)	0.77	57.3	1.2	143
GGM- <i>g</i> -C14 (1.14)	ND ^a	<51.2	1.6	102
GGM- <i>g</i> -C14 (1.95)	0.78	51.1	1.8	94
GGM- <i>g</i> -C18 (0.68)	ND ^a	<56.8	1.4	117
GGM- <i>g</i> -C18 (1.07)	ND ^a	<61.3	0.8	214
GGM- <i>g</i> -C18 (1.42)	ND ^a	<61.0	0.9	175
GGM- <i>b</i> -C9	2.44	44.5	2.3	73
GGM- <i>b</i> -C14	1.82	47.11	1.4	111
GGM- <i>b</i> -C18	0.88	49.5	1.7	96
^a not determined				

The block-structured GGM-fatty acid derivatives were all soluble in water at high concentrations. The measured CAC values decreased from GGM-*b*-C9 to GGM-*b*-C18 while γ_{\min} increased (Table 2). Taking the determined surface excess values into account, it can be assumed that for these products, not the GGM part but rather the hydrophobic chains are the limiting factor. This might be due to a denser packing of the short C9 chains at the surface compared to the longer C14 and C18 chains. The GGM chains are more flexible in the block-structured products and can arrange themselves in a more space-saving way compared to the grafted products. For the GGM derivatives of pelargonic acid (C9) and myristic acid (C14), the block-structured derivatives showed similar γ_{\min} values as the respective grafted product, with a FA/GGM ratio around 2. For the GGM-C18 derivatives, only the block-structured products had a sufficient solubility to show a significant surface activity.

The results demonstrated that it is possible to build amphiphilic GGM-fatty acid derivatives. This opens a research window in the future to investigate carbohydrate-based surfactants. Future work will focus on more surface-active derivatives, *e.g.*, by designing star-shaped GGM derivatives with two (or more) fatty acid chains attached to the reducing end.

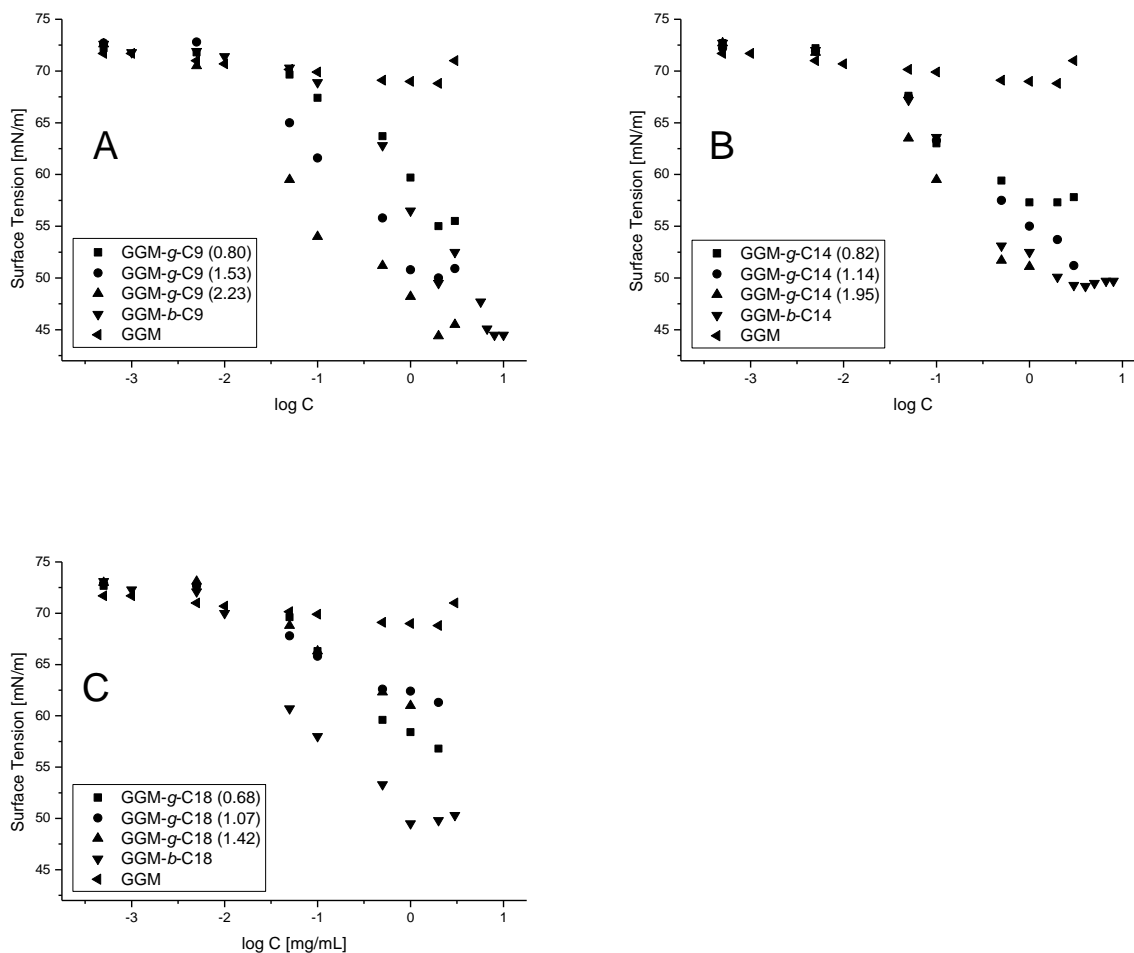


Fig. 8. Surface tension isotherms for GGM derivatives of pelargonic acid (C9 (A)), myristic acid (C14 (B)), and stearic acid (C18 (C))

CONCLUSIONS

1. Non-ionic carbohydrate-based surfactants can be synthesised from spruce GGM using naturally-occurring fatty acids.
2. Novel block and grafted amphiphilic macromolecules can be prepared using different synthesis routes. Esterification and reductive amination can be performed in homogeneous conditions using different solvent mixtures.
3. GGM derivatives grafted with fatty acids with 0.68 to 5.45 fatty acids per GGM chain can be prepared by esterification. Amino-functional fatty acids can be coupled to the reducing end of GGM to form block-structured amphiphilic GGM derivatives.
4. The resulting amphiphiles exhibited promising surfactant properties and can find potential applications in such areas as food, cosmetics, or water-based paints.

- Moreover, this study has demonstrated a novel approach to utilise naturally-occurring materials, *e.g.*, plant polysaccharides and fatty acids, for the development of highly value-added materials to replace petroleum-based substances.

ACKNOWLEDGMENT

Daniel Dax thanks the National Research Fund of Luxembourg (AFR) for granting him a scholarship. The Graduate School of Biomass Refining (BIOREGS) is thanked for financial support. This work was also part of the activities at the Åbo Akademi Process Chemistry Centre.

REFERENCES CITED

- Al Manasrah, M., Kallioinen, M., Ilvesniemi, H., and Mänttari, M. (2012). "Recovery of galactoglucomannan from wood hydrolysate using regenerated cellulose ultrafiltration membranes," *Bioresour. Technol.* 114, 375-381.
- Bernard, J., Save, M., Arathoon, B., and Charleux, B. (2008). "Preparation of a xanthate-terminated dextran by click chemistry: Application to the synthesis of polysaccharide-coated nanoparticles via surfactant-free ab initio emulsion polymerization of vinyl acetate," *J. Polym. Sci., Part A: Polym. Chem.* 46(8), 2845-2857.
- Carbonero, R. C., Mellinger, C. G., Eliasaro, S., Gorin, P. A. J., and Iacomini, M. (2005). "Chemotypes significance of lichenized fungi by structural characterization of heteropolysaccharides from the genera *Parmotrema* and *Rimelia*," *Microbiol. Letters* 246(2), 273-278.
- Cho, H. S., Moon, H. S., Lim, J. Y., and Kim, J. Y. (2013). "Effect of long chain fatty acids removal as a pretreatment on the anaerobic digestion of food waste," *J. Mater. Cycles Waste Manag.* 15(1), 82-89.
- Ferrer, M., Comelles, F., Plou, F. J., Cruces, M. A., Fuentes, G., Parra, J. L., and Ballesteros, A. (2002). "Comparative surface activities of di- and trisaccharide fatty acid esters," *Langmuir* 18(3), 667-673.
- Garofalakis, G., Murray, B. S., and Sarney, D. B. (2000). "Surface activity and critical aggregation concentration of pure sugar esters with different sugar headgroups," *J. Colloid Interface Sci.* 229(2), 391-398.
- Grefe, L., Bessueille, L., Bulone, V., and Brumer, H. (2005). "Synthesis, preliminary characterization, and application of novel surfactants from highly branched xyloglucan oligosaccharides," *Glycobiology* 15(4), 437-445.
- Grote, C., and Heinze, T. (2005). "Starch derivatives of high degree of functionalization 11: Studies on alternative acylation of starch with long-chain fatty acids homogeneously in *N,N*-dimethyl acetamide/LiCl," *Cellulose* 12(4), 435-444.
- Halila, S., Manguian, M., Fort, S., Cottaz, S., Hamaide, T., Fleury, E., and Driguez, H. (2008). "Syntheses of well-defined glycol-polyorganosiloxanes by "click" chemistry and their surfactant properties" *Macromol. Chem. Phys.* 209(12), 1282-1290.
- Hannuksela, T., and Penhoat, C. H. (2004). "NMR structural determination of dissolved O-acetylated galactoglucomannan isolated from spruce thermomechanical pulp," *Carbohydr. Res.* 339(2), 301-312.

- Hartman, J., Albertsson, A. -C., and Sjöberg, J. (2006). "Surface- and bulk-modified galactoglucomannan hemicellulose films and film laminates for versatile oxygen barriers," *Biomacromolecules* 7(6), 1983-1989.
- Hennink, W. E., and van Nostrum, C. F. (2002). "Novel crosslinking methods to design hydrogels," *Adv. Drug Delivery Rev.* 54(1), 13-36.
- Houga, C., Le Meins, J. -F., Borsali, R., Taton, D., and Gnanou, Y. (2007). "Synthesis of ATRP-induced dextran-*b*-polystyrene diblock copolymers and preliminary investigation of their self-assembly in water," *Chem. Commun.* 43(29), 3063-3065.
- Hussain, M. A., Liebert, T., and Heinze, T. (2004). "Acylation of cellulose with *N,N'*-carbonyldiimidazole-activated acids in the novel solvent dimethyl sulfoxide/tetrabutylammonium fluoride," *Macromol. Rapid Commun.* 25(9), 916-920.
- Jönsson, B., Lindman, B., Holmberg, K., and Kronberg, B. (1998). *Surfactants and Polymers in Aqueous Solutions*, Wiley, West Sussex.
- Kisonen, V., Eklund, P., Auer, M., Sjöholm, R., Pranovich, A., Hemming, J., Sundberg, A., Aseyev, V., and Willför, S. (2012). "Hydrophobication and characterisation of O-acetyl-galactoglucomannan for papermaking and barrier applications," *Carbohydr. Res.* 352, 151-158.
- Lahaye, M., and Robic, A. (2007). "Structure and functional properties of ulvan, a polysaccharide from green seaweeds," *Biomacromolecules* 8(6), 1765-1774.
- Leppänen, A. -S., Niittymäki, O., Parikka, K., Tenkanen, M., Eklund, P., Sjöholm, R., and Willför, S. (2010). "Metal-mediated allylation of enzymatically oxidized methyl α -D-galactopyranoside," *Carbohydr. Res.* 345(18), 2610-2615.
- Lindqvist, H., Holmback, J., Rosling, A., Salminen, K., Holmbom, B. Auer, M., and Sundberg, A. (2013). "Galactoglucomannan derivatives and their application in papermaking," *BioResources* 8(1), 994-1010.
- Michielsen, S., Brandrup, J., and Immergut, E. H. (1999). *Polymer Handbook*, 4th Ed., Wiley, New York.
- Moure, A., Gullón, P., Domínguez, H., and Parajó, J. C. (2006). "Advances in the manufacture, purification and applications of xylo-oligosaccharides as food additives and nutraceuticals," *Process Biochem.* 41(9), 1913-1923.
- Peterson, K., Nielsen, P.V., Bertelson, G., Lawther, M., Olsen, M.B., Nilsson, N. H., and Mortensen, G. (1999). "Potential of biobased materials for food packaging," *Trends Food Sci. Tech.* 10(2), 52-68.
- Sadhu, V. B., Pionteck, J., Voigt, D., Komber, H., and Voit, B. (2004). "Synthesis of halogen-free amino-functionalized polymethyl methacrylate by atom transfer radical polymerization (ATRP)," *Macromol. Symp.* 210(1), 147-155.
- Taghizadeh, S. M., and Davari, G. (2006). "Preparation, characterization, and swelling behavior of N-acetylated and deacetylated chitosans," *Carbohydr. Polym.* 64(1), 9-15.
- Voepel, J., Edlund, U., and Albertsson, A.-C. (2011). "A versatile single-electron-transfer mediated living radical polymerization route to galactoglucomannan graft-copolymers with tunable hydrophilicity," *J. Polym. Sci., Part A: Polym. Chem.* 49(11), 2366-2372.
- Willför, S., Rehn, P., Sundberg, A., Sundberg, K., and Holmbom, B. (2003a). "Recovery of water-soluble acetylgalactoglucomannans from mechanical pulp of spruce," *Tappi J.* 2(11), 27-32.
- Willför, S., Sjöholm, R., Roslund, M., Hemming, J., and Holmbom, B. (2003b). "Characterisation of water-soluble galactoglucomannans from Norway spruce wood and thermomechanical pulp," *Carbohydr. Polym.* 52(2), 175-187.

- Willför, S., Sundberg, K., Tenkanen, M., and Holmbom, B. (2008). "Spruce-derived mannans – A potential raw material for hydrocolloids and novel advanced natural materials," *Carbohydr. Polym.* 72(2), 197-210.
- Xu, C., Willför, S., Holmlund, P., and Hombom, B. (2009). "Rheological properties of water-soluble spruce O-acetyl galactoglucomannans," *Carbohydr. Polym.* 75(3), 498-504.
- Xu, C., Willför, S., and Holmbom, B. (2008). "Rheological properties of mixtures of spruce galactoglucomannans and konjac glucomannan or some other polysaccharides," *BioResources* 3(3), 713-730.
- Zhang, T., and Marchant, R. E. (1994). "Novel polysaccharide surfactants: Synthesis of model compounds and dextran-based surfactants," *Macromolecules* 27(25), 7302-7308.
- Zhang, T., and Marchant, R. E. (1996). "Novel polysaccharide surfactants: The effect of hydrophobic and hydrophilic chain length on surface active properties," *J. Colloid Interface Sci.* 177(2), 419-426.

Article submitted: April 21, 2013; Peer review completed: May 27, 2013; Revised version received and accepted: May 29, 2013; Published: May 30, 2013.