Unconventional Methyl Galactan Synthesized via the Thexyldimethylsilyl Intermediate: Preparation, Characterization, and Properties

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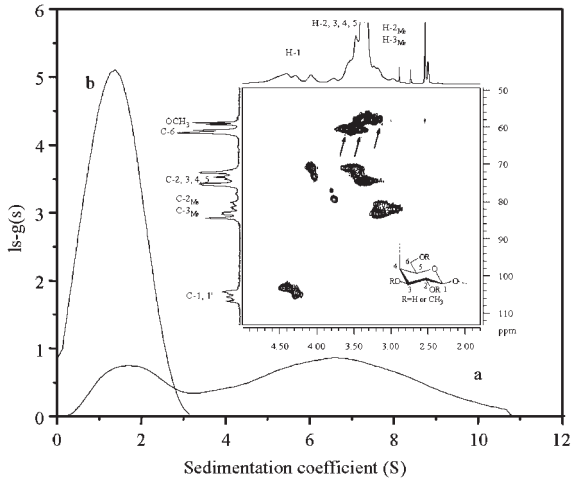
Reaction of a β-(1 $\rightarrow$ 4) linked galactan with TDMS chloride followed by methylation and desilylation yields methyl galactans with unconventional functionalization patterns. The products were characterized via FTIR and NMR of the intact polymer and by CE after controlled depolymerization. A TDMS-derivatized methyl galactan contains differently methylated secondary hydroxyl groups. SEC and analytical ultracentrifugation showed a consistent decrease in the molecular weight after the consecutive reaction steps. Biological studies revealed that the methyl galactans are less active in complement fixation assays as compared with a 3-*O*-methyl galactan-enriched polysaccharide fraction isolated from *Acanthus ebracteatus*.



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Introduction

For quite some time, medicinal plants, such as *Acanthus ebracteatus* Vahl, have been used in the treatment of different diseases including hepatitis, lymphoma, and asthma.[1–3] Extracts also possess anti-tumor and anti-mutagenic activities.[4,5] Extraction, fractionation, and physico-chemical characterization of the polysaccharides in *Acanthus ebracteatus* Vahl have shown that fractions containing 3-*O*-methyl galactose are highly active towards the complement system.[6] Sugar analysis revealed the presence of up to 55% β-(1 $\rightarrow$ 4) linked galactose and up to

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33% 3-*O*-methyl galactose in the main chain. It has therefore been assumed that (1 → 4) linked 3-*O*-methyl galactose is of vital importance for the activity measured. However, the knowledge of galactan functionalization is quite limited. Derivatives of galactan, such as the methyl ether, have only been prepared for analytical purposes in order to determine the sugar composition and linkage types of polymer chains.[7] In addition, sulfuric acid half esters of galactan have been synthesized. These exhibit an increased bioactivity as compared with the original polysaccharide.[8–10] Regioselective functionalizations of galactan have not been published to date.

In contrast to this, efficient methods have been developed for regioselective functionalization of cellulose and starch by employing bulky triphenylmethyl ethers or trialkylsilyl ethers as protecting groups.[11,12] For example, use of the thexyldimethylsilyl (TDMS) group allows the synthesis of both 6-*O*-protected and 2,6-di-*O*-protected cellulose and starch derivatives by simply varying the reaction conditions.[13] In an extension of this strategy, the TDMS group has recently been employed to synthesize 3-*O*-functionalized cellulose ethers.[14–16]

Working under the assumption that methylation at position 3 is essential for the bioactivity of methyl galactans, we now present the first specific functionalization studies on a β-(1 → 4) galactan. Reaction of β-(1 → 4) galactans with TDMS–Cl under homogeneous reaction conditions followed by methylation and desilylation yields various methylated compounds. These were characterized with respect to their structures, molecular weights, sedimentation coefficient distributions, and biological activities.

Experimental Part

Materials

The galactan used for the investigations was purchased from Megazyme (Bray, Ireland). Thexyldimethylchlorosilane (TDMS–Cl) was obtained from ABCR (Karlsruhe, Germany). Sodium hydride (suspension in mineral oil, Fluka, Taufkirchen, Germany) was washed with hexane and pentane and dried in vacuum at room temperature prior to use. Galactan was dried over potassium hydroxide in vacuum at 105 °C and LiCl was dried over potassium hydroxide in vacuum at 150 °C. All other chemicals (Sigma Aldrich, Munich, Germany) were used as received. Phosphate buffer solution (pH = 7) contained 7.14 $g \cdot L^{-1}$ K_2HPO_4 and 3.54 $g \cdot L^{-1}$ KH_2PO_4.

Preparation of Thexyldimethylsilyl Galactan 2

Galactan **1** (2.0 g, 12.3 mmol) was slurried in 80 mL of *N,N*-dimethylacetamide (DMA) and stirred for 2 h at 120 °C in a moisture-free environment. After cooling down to 100 °C, 4.8 g LiCl was added and stirring was continued without further heating until the polymer dissolved completely. After addition of 4.03 g of imidazole (59.2 mmol, 4.8 mol per mol anhydrogalactose unit), 8.8 g of TDMS–Cl (49.4 mmol, 4 mol per mol anhydrogalactose unit) was added slowly and the mixture was allowed to stir for 48 h at 100 °C. The supernatant was decanted followed by dissolution of the polymer in tetrahydrofuran (THF). After precipitation in ethanol, the polymer was washed with ethanol and dried at 60 °C in vacuum.

Yield: 3.34 g (62%).

Elemental analysis: SiO_2 26.71% (Degree of substitution (DS) = 1.96)

IR (KBr): 3 601, 3 457 (OH), 2 960, 2 872 (CH), 1 754 (C=O), 1 467 (CH), 1 379 (CH), 1 255 (SiC), 1 106, 1 059 (COC_{AGU}), 833, 777 cm^{-1} (SiC).

The polymer was soluble in chloroform, THF, and toluene.

Preparation of Methyl Thexyldimethylsilyl Galactan 3

Thexyldimethylsilyl galactan **2** (2.78 g, 6.23 mmol, DS = 1.96) was dissolved in 80 mL of dry THF in a moisture-free environment. Sodium hydride (1.49 g, 62.08 mol, 10 mol per mol modified anhydrogalactose unit) was carefully added followed by 8.84 mL of methyl iodide (62.08 mol, 10 mol per mol modified anhydrogalactose unit). The mixture was allowed to stir for 1 d at room temperature and 3 days at 50 °C. After precipitation in phosphate buffer solution (pH = 7) and filtration, the polymer was washed with water and dried at 60 °C in vacuum.

Yield: 2.33 g.

IR (KBr): 3 441 (w, OH), 2 957, 2 871 (CH), 1 465, 1 377 (CH), 1 251 (SiC), 1 112, 1 063, 1 036 (COC_{AGU}), 834, 776 cm^{-1} (SiC).

The polymer was soluble in chloroform, hexane, THF, and toluene.

Preparation of Methyl Galactan 4

Methyl thexyldimethylsilyl galactan **3** (2.0 g, 4.34 mmol, based on DS_{Si} 2, DS_{Me} 1) was dissolved in 40 mL of THF and stirred with 5.49 g of tetrabutylammonium fluoride trihydrate ($TBAF \cdot 3H_2O$, 17.4 mmol, 2 mol per mol silyl group) for 24 h at 50 °C. The THF was decanted and isopropyl alcohol was added to solidify the polymer, which was washed with isopropyl alcohol, dissolved in water and freeze-dried to yield 0.57 g of polymer. ^{1}H NMR spectroscopy revealed incomplete desilylation. Thus, the sample was dissolved in dimethylsulfoxide (DMSO) and was allowed to react with 1.0 g of $TBAF \cdot 3H_2O$ for 24 h at 50 °C. Work-up was carried out as described above to yield 0.46 g of polymer, which still contained TDMS moieties. The desilylation in DMSO solution was repeated.

Yield: 0.4 g.

^{1}H NMR (DMSO-d_6): δ = 0.09–1.57 (TDMS moiety), 2.73–4.42 (modified anhydrogalactose).

^{13}C NMR (DMSO-d_6): δ = 13.9 (tetrabutylammonium, TBA), 18.9 (TDMS, TBA), 19.7 (TBA), 20.6 (TBA), 25.0 (TBA), 33.3 (TDMS), 36.2 (TDMS), 58.0, 58.6 (TBA, OCH_3), 60.1 (OCH_3), 60.7 (C-6), 71.5–79.5

(C-2, 3, 4, 5), 80.9–83.8 (C-2_s, C-3_s), 103.6, 104.9, 106.1 (C-1, C-1′), 162.8 (C=O).

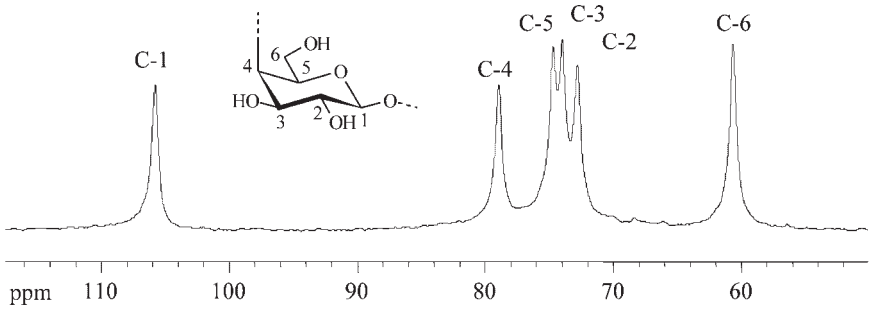

Figure 1. ^{13}C NMR spectrum of β-(1→4) galactan from Lupine seeds recorded in DMSO-d_6 (40 °C, 163 840 scans).

Measurements

FTIR spectra were acquired with a Nicolet AVATAR 370 DTGS spectrometer in KBr. NMR spectra were obtained with a Bruker Avance 400 spectrometer in DMSO-d_6 (5%) at 40 °C using standard pulse sequences for ^1H, ^{13}C, DEPT 135, and two-dimensional (COSY, HSQC/DEPT) NMR spectra. The scan number was 16 for ^1H and up to 163 840 for ^{13}C NMR spectra.

A Beckman P/ACE DNA System (Beckman, Munich, Germany) was employed for capillary electrophoresis, working with a fused silica capillary (length 50/57 cm, 50 μm inner diameter, Beckman, Munich, Germany) with a voltage of 28 kV at 25 °C. Ultraviolet (UV) detection was carried out at 285 nm and P/ACE Station software (Beckman, Munich, Germany) was used for processing. The system was operated with 150×10^{-3} M borate buffer at pH = 10. The sample preparation including hydrolysis and derivatization with 4-aminobenzoic acid was carried out according to Tüting et al.[17]

A Jasco size exclusion chromatography (SEC) system used for SEC measurements consisted of a degasser DG 980-50, pump PU 980, UV detector 975 (λ = 254 nm), refractive index detector 930, and a column oven working at 30 °C. Sodium azide (0.05%) was used as eluent at a flow rate of 1 mL · min^{-1}. The columns PLaqua 60, PLaqua 50, and PLaqua 40 were purchased from Polymer Laboratories (Darmstadt, Germany).

The intrinsic viscosities were determined with an automatic viscometer (Lauda PVS 1/2) equipped with a dilution Ubbelohde viscometer (capillary No. I, Schott Instruments, Mainz, Germany) in a thermostated water bath (Lauda E 200, Lauda-Königshofen, Germany) at 20 °C. An automatic burette (Metrohm Dosimat 765, Filderstadt, Germany) was used to dilute the solutions automatically.

Sedimentation velocity experiments were performed using a Beckman Instruments (Palo Alto, USA) Optima XLI analytical ultracentrifuge. Galactan **1** and methyl galactan **4** solutions (380 μL, 3 mg · mL^{-1}) and distilled water (400 μL) were injected into the solution and reference channels, respectively, of a double sector 12-mm optical path length cell. Samples were centrifuged at 45 000 rpm at 20.0 °C. Concentration profiles and the movement of the sedimenting boundary in the analytical ultracentrifuge cell were recorded using the Rayleigh interference optical system and converted to concentration (in units of fringe displacement relative to the meniscus, *j*) versus radial position, *r*.[18] The data was then analyzed using the ls-g(s) model incorporated into the SEDFIT (Version 10.09beta) program.[19,20] This software based on the numerical solutions to the Lamm equation follows the changes in the concentration profiles with radial position and time and generates an apparent distribution of sedimentation coefficients in the form of $g^*(s)$ versus $s_{20,w}$, where the asterisk indicates that the distribution of sedimentation coefficients has not been corrected for diffusion effects. Sedimentation velocity has been utilized previously to assess changes in sedimentation coefficients (and their distributions) after the chemical functionalization of a heteroxylan[21] and low methoxyl pectins.[22]

Complement Fixation Test

The complement fixation test is based on inhibition of hemolysis of antibody sensitized sheep red blood cells by the complement from human sera as described by Michaelsen et al. (method A).[23] Veronal buffer/bovine serum albumin, serum, and sensitized sheep erythrocytes were the control of the medium, and the pectin fraction PM II from the leaves of *Plantago major* was used as positive control.[24]

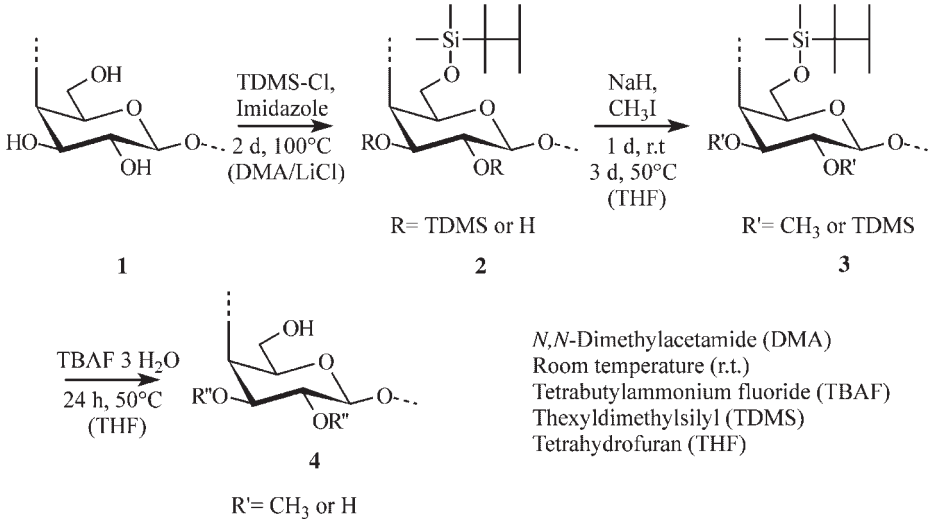

Figure 2. Reaction scheme for the preparation of methyl galactan with unconventional functionalization pattern via galactan thexyldimethylsilyl ether.

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Results and Discussion

Synthesis and Structural Characterization

Galactan **1** was extracted from Lupine seeds and subsequently treated with α-L-arabinofuranosidase to remove the bulk of the arabinose units. The polymer obtained by this method is composed mainly of galactose (83%). To a lesser degree, it also contains galacturonic acid (5%), rhamnose (5%), arabinose (3%), xylose (2%), and traces of glucose. The ^{13}C NMR spectrum of **1** recorded in DMSO-d_6/LiCl clearly showed the expected resonances of the galactan backbone at 60.7 (C-6), 72.8 (C-2), 74.0 (C-3), 74.7 (C-5), 78.9 (C-4), and 105.8 ppm (C-1) (Figure 1, peak assignments according to Vogl et al.[10]). Even after high accumulation of scans (163 840), signals originating from other sugars were not observed in the spectrum.

Galactan **1** is quite soluble in DMA after the addition of LiCl. Solutions thus prepared were reacted with 4 mol of TDMS–Cl in the presence of 4.8 mol of imidazole for 48 h at 100 °C (Figure 2). In the course of this conversion, the TDMS-galactan formed precipitated from the solution. A product with DS 1.96 (sample **2**) was isolated and was soluble in THF, chloroform, and toluene.

The FTIR spectrum of **2** showed typical absorption bands at 3 601, 3 457 (ν_{OH}), 2 960, 2 872 (ν_{CH}), 1 754 ($\nu_{C=O}$), 1 467 (δ_{CH}), 1 379 (δ_{CH}), 1 255 (δ_{SiC}), 1 106, 1 059 ($\nu_{COC,\ AGU}$), 833, and 777 cm^{-1} (ν_{SiC}). The DS approaches 2, which indicates that there is no difference as compared to the conversion of cellulose. Assuming that the selectivity of the TDMS group towards galactan is comparable with cellulose, the OH groups at positions 2 and 6 might be blocked.

Reaction of **2** with excess methyl iodide in the presence of sodium hydride yielded the corresponding methylated TDMS-galactan **3**. The absorption bands at 2 958 and 2 871 (ν_{C-H}), 1 465 (δ_{C-H}), 1 111–1 036 cm^{-1} (ν_{C-O-C}) were attributed to the polymer backbone. Intensive bands at 1 251, 834, and 776 cm^{-1} were caused by the TDMS group. Weak bands at 3 441 and at 1 620 cm^{-1} belong to the ν_{OH} and $\nu_{C=O}$ of the galacturonic acid moieties.

Desilylation could be achieved by three consecutive treatments of solutions of **3** (dissolved in THF and DMSO) with TBAF · 3H$_2$O for 24 h at 50 °C. The product (methyl galactan **4**) was soluble in DMSO and cold water. In contrast to this, galactan **1** is only soluble in hot water or DMSO after addition of LiCl.

The ^{1}H NMR spectrum of **4** recorded in DMSO-d_6 was badly resolved but indicated the presence of peaks in the range between −1 and 1.6 ppm, which we assigned to TDMS moieties and TBA ions (Figure 3(a)). The TBA ion may act as a counter ion for the carboxylic acid groups of galacturonic acid. As has been reported, complete removal of the TDMS group may not be achieved for methylated polysaccharides.[14] The peaks of the modified repeating unit appeared between 2.8 and 5.0 ppm. The ^{13}C NMR spectrum of **4** (Figure 3(b)) recorded in DMSO-d_6 showed significant changes when compared with the ^{13}C NMR spectrum of **1**. The peaks of the modified repeating unit were detected between 58 and 105 ppm. In addition, a weak signal at 162.8 ppm is characteristic for the carboxylic acid group of galacturonic acid.

A more detailed peak assignment was achieved using HSQC/DEPT experiments (Figure 4). In the range from 57 to 61 ppm, different signals appeared that belong to both methoxy and CH_2 moieties (position 6) of the anhydrogalactose unit. Methoxy groups were detected at 58.0/3.62 ppm, 58.5/3.38 ppm, and 60.1/3.47 ppm. The main

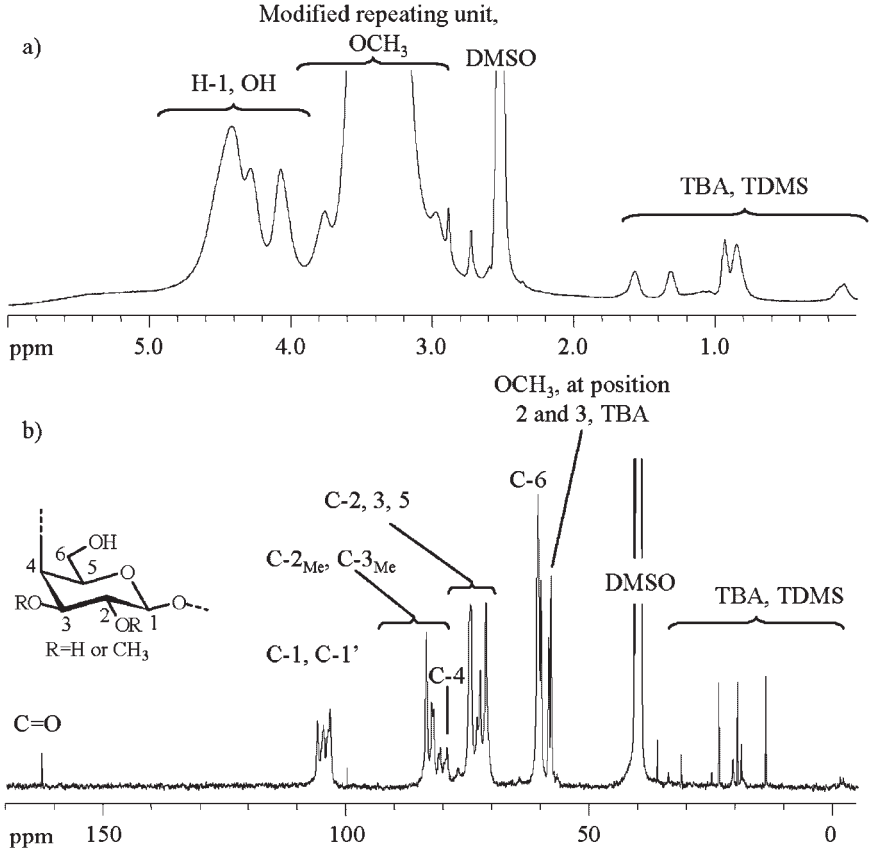

Figure 3. (a) ^{1}H and (b) ^{13}C NMR spectrum of methyl galactan **4** recorded in DMSO-d_6 at 40 °C (TBA, tetrabutylammonium).

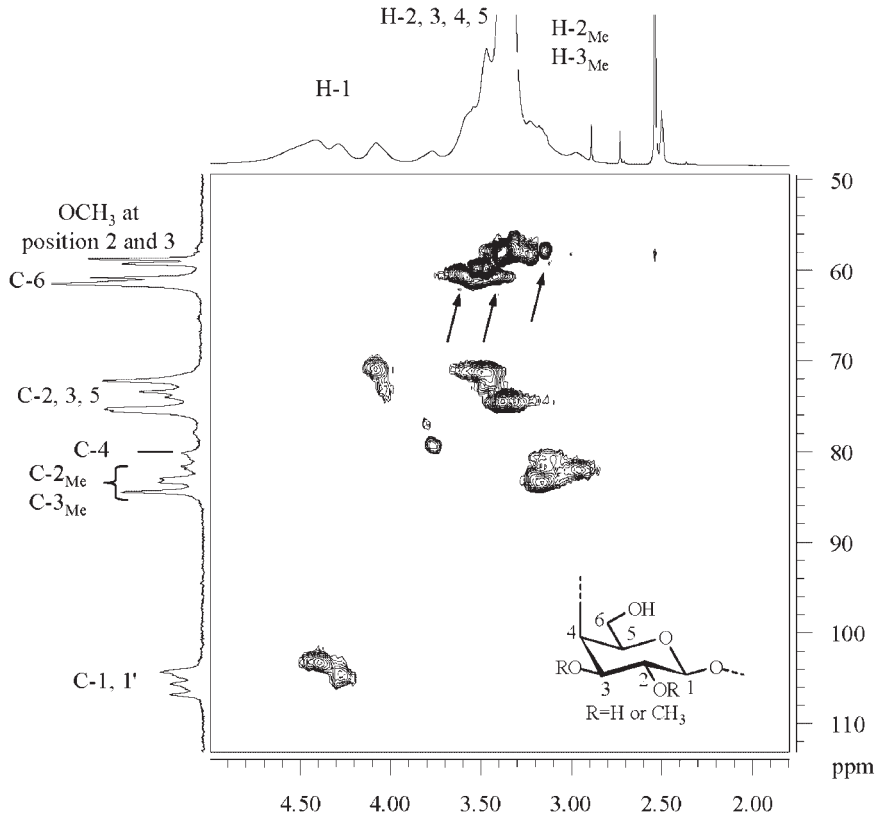

Figure 4. HSQC/DEPT NMR spectrum of methyl galactan **4** recorded in DMSO-d_6 at 40 °C. Methylene groups are marked by arrows.

signal of position 6 was detected at 60.8/3.59 ppm. It could not be clearly distinguished whether this is a CH_2OH or CH_2OCH_3 group. Moreover, a comparably weak resonance at 58.0/3.15 ppm corresponds to the methylene group of the TBA ion. This signal was split due to the neighboring ^{14}N atom. The peaks in the range from 70/3.3 to 80/4.1 ppm were caused by the positions 2–5 of the modified anhydrogalactose unit. The methylation of the secondary OH groups led to a significant downfield shift of the carbon resonances. These signals appeared between 80.0/2.8 and 84.7/3.3 ppm.

It is well known that the chemical shift of the anomeric carbon atom is influenced by substituents at position 2. In contrast to galactan **1**, where only one signal was detected, in the ^{13}C NMR spectrum of **4**, three peaks between 101.9/4.2 and 106.3/4.5 ppm were observed, which indicate a functionalization pattern at position 2. The acquisition of a better resolved spectrum would require a decrease in the intramolecular and intermolecular interactions of the polymer chain. This can usually be achieved by polymer peracylation. However, in this case, a low extent of cross-linkage with subsequent insolubility due to the presence of carboxylic acid moieties occurred.

The functionalization pattern in **4** was then investigated by means of capillary electrophoresis (CE) after controlled depolymerization. Preliminary results showed a CE curve for **4** that contained a set of different signals at a retention time between 4.5 and 5.5 min (Figure 5). Co-injection of rhamnose, xylose, arabinose, glucose, and galacturonic acid revealed that these signals were caused by methylated sugars. Differently functionalized repeating units are present in the polymer chain in addition to a small quantity of galactose (retention time 7.3 min). Due to the lack of standard compounds, these peaks could not be assigned in detail. However, the CE results support the NMR spectroscopic results; that is, methylation has occurred not only in position 3.

This unexpected regioselectivity of the galactan has several possible origins. The silylation could occur selectively as has been reported for comparable conversions of cellulose and starch; that is, positions 2 and 6 have been completely modified. A detailed investigation of carbohydrate *tert*-butyldimethylsilyl (TBDMS) ether has demonstrated that silyl ethers migrate under strong alkaline conditions.[25] In addition, migration of the TBDMS ether from position 2 to position 3 was reported for a methyl

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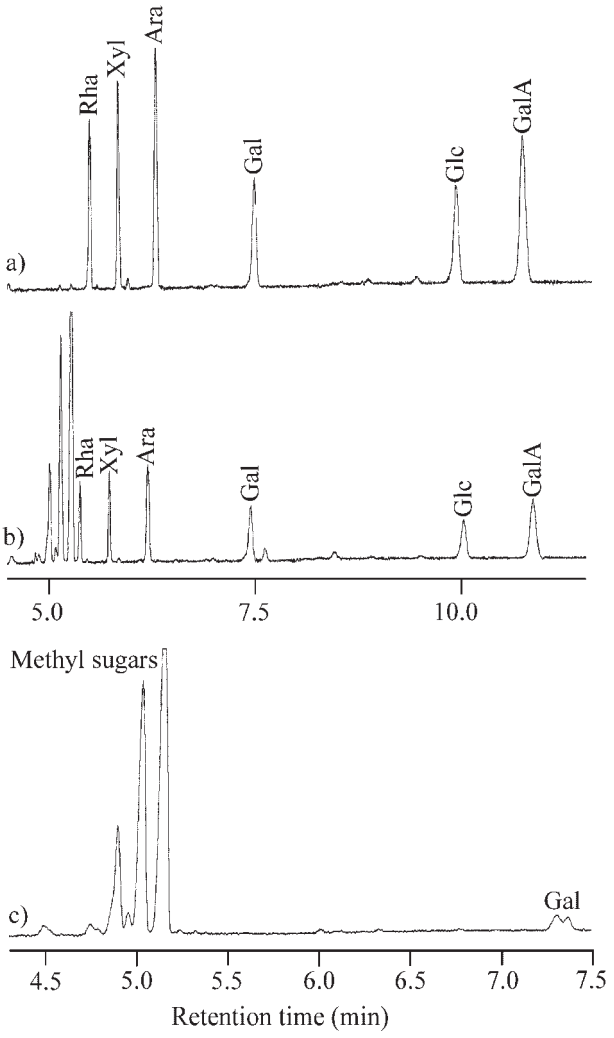

Figure 5. Capillary electrophoresis curves of (a) a sugar standard containing rhamnose (Rha), xylose (Xyl), galactose (Gal), glucose (Glc), and galacturonic acid (GalA), (b) the methyl galactan **4** after depolymerization and derivatization mixed with the sugar standard, and (c) the methyl galactan **4** after depolymerization without standard.

4, 6-benzylidene-2-*O*-TBDMS-β-D-galactopyranoside.[26] The same behavior was found in TBDMS ethers of cyclodextrins.[27]

The TDMS group employed in this study may exhibit similar properties. However, additional studies, except for a single investigation reporting the 1,2-migration of the TDMS group in a 4′,6′-benzylidene lactoside,[28] have not been reported in the chemical literature. In the case of polysaccharides, a rearrangement was found during the conversion of 2,6-di-*O*-TDMS starch with methyl iodide in the presence of sodium hydride, whereas methylation of 2,6-di-*O*-TDMS cellulose led to the corresponding 3-*O*-methyl ether. This effect was explained with the α-configuration of the glycosidic linkage (Figure 6(a), according to Mischnick et al.[29]). The deprotonated OH group of position 3 is believed to attack the silyl ether at position 2 to form a cyclic intermediate that subsequently opens, thus shifting the TDMS group from position 2 to position 3. Therefore, the corresponding 2-*O*-methyl ether may be present after methylation and desilylation. A similar mechanism may occur in the case of the β-(1 → 4) linked galactan (Figure 6(b)).

Another explanation may be that the structure of the galactan prevents selective introduction of the TDMS ether. This would result in non-selective methylation of the polymer. Figure 7 shows the result of a rough molecular dynamics simulation of β-(1 → 4) galactan, cellulose, and starch surrounded by water. All polymer chains formed somewhat helical structures. However, due to the β-configuration of the glycosidic bond at position 1 and the axial position of the glycosidic bond at position 4 of the repeating unit, the flexibility of galactan seemed to be superior to that of cellulose and starch. This led us to conclude that steric hindrance during silylation can be compensated by a conformational change; that is, the formation of the TDMS ether is not position selective at either position 2 or position 6.

Characterization of the Molar Mass Distribution

SEC measurements in aqueous solution revealed a bimodal distribution in the molar mass distribution for galactan **1** with maxima at 17 000 and 440 000 g · mol^{-1} (Figure 8(a)). The sharp bend of the curve at 1 600 000 g · mol^{-1} was caused by a discontinuity of the SEC calibration curve. The SEC curve of **4** exhibited a unimodal distribution of the molar mass with a maximum at 70 000 g · mol^{-1} (Figure 8(b)). The molar mass was significantly reduced compared to galactan **1**. This could be caused by the extensive purification required by this multi-step synthesis. The carboxylic acid groups of the galacturonic acid moieties may promote hydrolytic depolymerization. It must also be taken into account that polysaccharides form aggregates in solution which contribute to the molar mass of the polymer. This aggregation may also be influenced by the DS. In other words, increasing the functionalization of the hydroxyl groups may decrease the tendency towards aggregation. A reduction in molar mass after chemical functionalization (carboxybenzylation) has been previously reported for low methoxyl pectin.[22]

The intrinsic viscosity [η] reflects the size of the macromolecule and depends on the chain rigidity as well as the quality of the solvent. It is known that neutral polysaccharides have a low hydrodynamic volume. Neutral sugar side chains do not contribute significantly to the intrinsic viscosity. The viscosity [η] is thus mainly influenced by the chain length.[30] From the Huggins plot

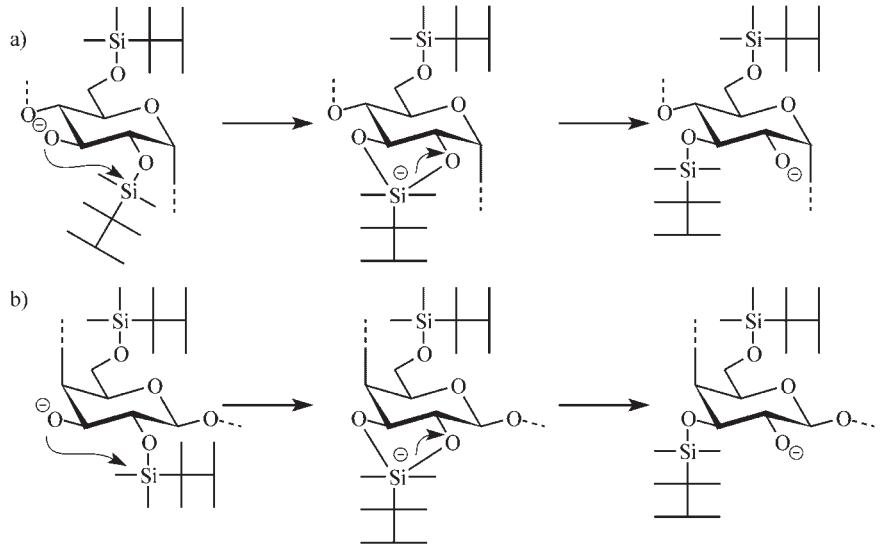

Figure 6. Rearrangement of thexyldimethylsilyl groups in amylose (a) during the methylation (according to Mischnick et al.[29]) and (b) a possible mechanism in β-(1 → 4) galactan.

of samples **1** and **4**, it was obvious that the molar mass had decreased during the synthesis (Figure 9). This is consistent with the findings reported for low methoxyl pectin.[22] It should be noted that, in the case of galactan **1,** the intrinsic viscosity is the average of both molar mass populations.

The ls-g(s) plots for galactan **1** and methyl galactan **4** were consistent with the results from SEC and viscometry experiments; that is, galactan **1** had a bimodal distribution (Figure 10(a)) ($s_{20,w} = 1.7$ and 6.5 S, respectively), whereas methyl galactan **4** showed a unimodal distribution ($s_{20,w} = 1.3$ S) (Figure 10(b)). Sedimentation coefficients also reflect the size of the macromolecule and the chain rigidity (although in the case of comparable molar masses, the more rigid macromolecule will have the lower sedimentation coefficient). We assume that the decrease in sedimentation coefficient after functionalization is due to depolymerization during synthesis. In accord with this, the decrease in sedimentation coefficient for a comparable system (carboxybenzylation of low methoxyl pectin) has been shown to be almost entirely due to the decrease in molar mass.[22]

Bioactivity

As illustrated in Figure 11, the effect of the original galactan and the methyl galactan on the complement fixation assay was negligible compared with bioactive polysaccharide PM II from *Plantago major* used as a standard. It has often been assumed that in order to have an effect in the complement assay, a multivalent type of binding must take place.[31] Since these polymers are relatively simple, unbranched, long-chain molecules, this could explain the lack of activity. In the 3-*O*-methyl galactose-rich polymers isolated from *Acanthus ebracteatus*, it appeared that the methyl galactans are linked to other types of polysaccharide moieties such as pectins. This could give rise to a complex three-dimensional structure leading to several binding sites in this macromolecule. If it would be possible to attach the synthesized methyl galactan to several sites on a pectic moiety, it would be of interest to find out if the activity of this polymer increased in the same way as we find for those polymers described by Hokputsa et al.[6]

Conclusion

In this study, we have succeeded in synthesizing a methyl galactan with an unconventional functionalization pattern. In addition, we have demonstrated that the result of this multi-step synthesis via the thexyldimethylsilyl ether differs from comparable conversions of cellulose. In contrast to the 3-*O*-functionalization observed for cellulose, both secondary OH groups in galactan were methylated to a certain degree. Further studies should be carried out in order to clarify this behavior. The novel methyl galactans obtained were comprehensively characterized using several different methods. Size exclusion chromatography and analytical ultracentrifugation gave consistent results. The biological activity of these compounds was lower than that of a 3-*O*-methyl galactan-rich

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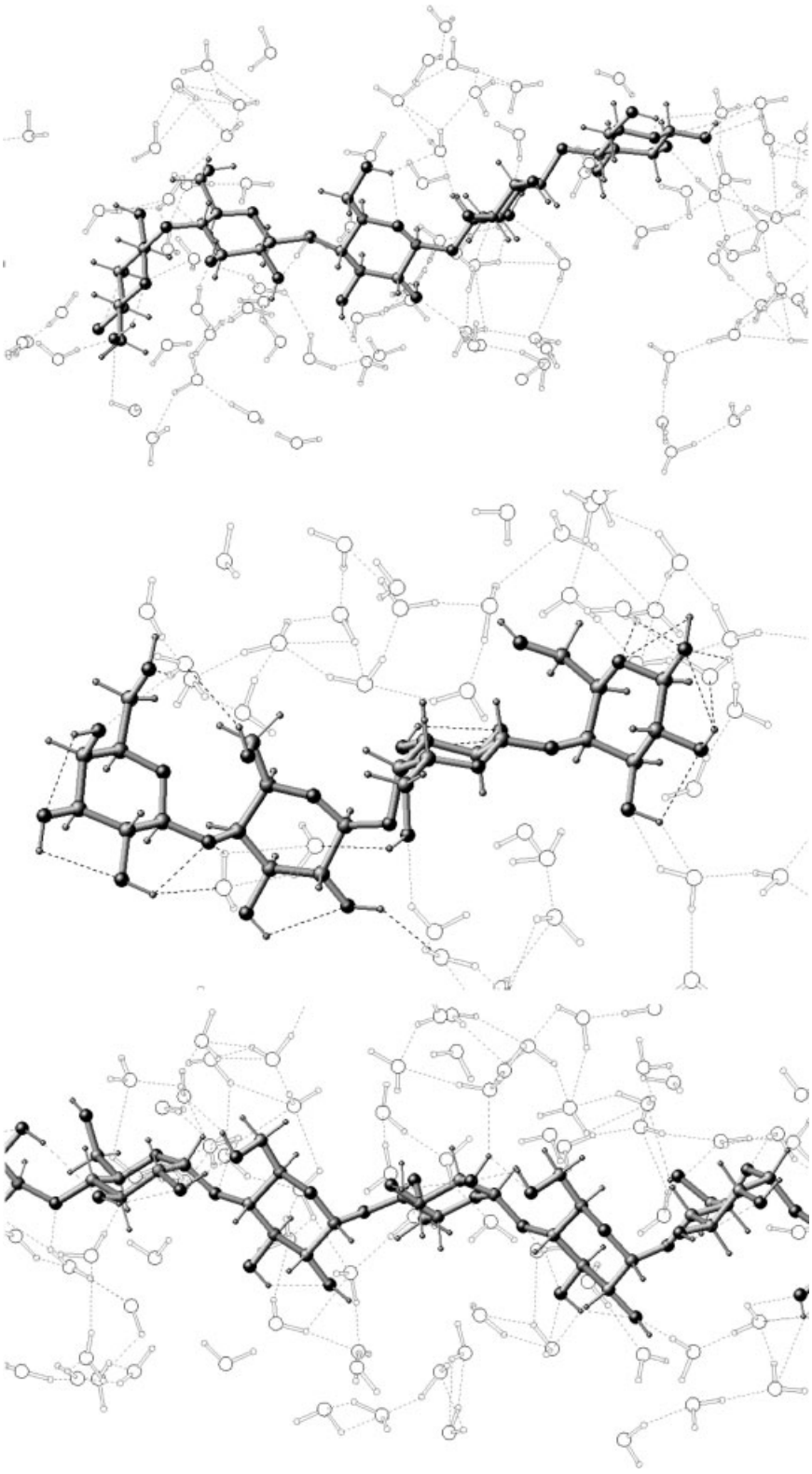

Figure 7. Molecular structures of cellulose (top), β-(1 $\rightarrow$ 4) galactan (middle), and amylose (bottom) surrounded by water after molecular dynamics simulation and geometry optimization.

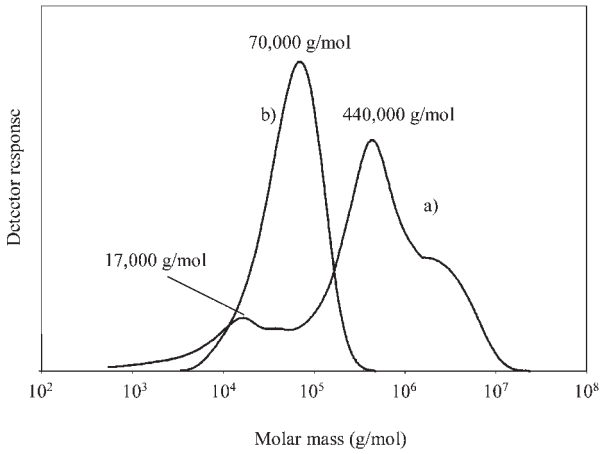

Figure 8. Size exclusion chromatograms of galactan **1** (a) and methyl galactan **4** (b) recorded in water.

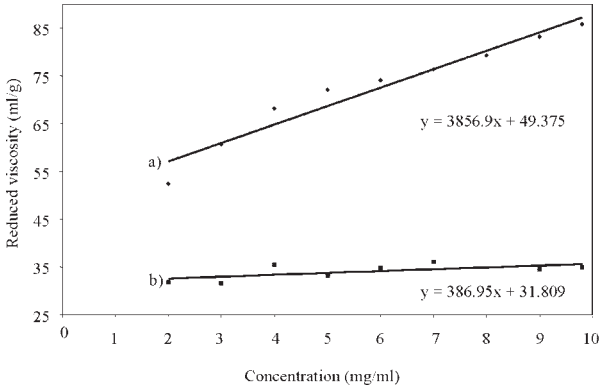

Figure 9. Huggins plot of reduced viscosity versus concentration of galactan **1** (a) and methyl galactan **4** (b) dissolved in water.

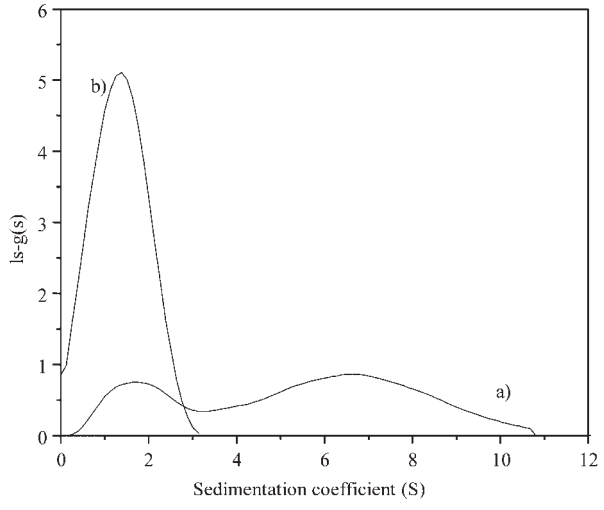

Figure 10. Sedimentation coefficient distributions [ls-g(s)] of galactan **1** (a) and methyl galactan **4** (b) recorded in water.

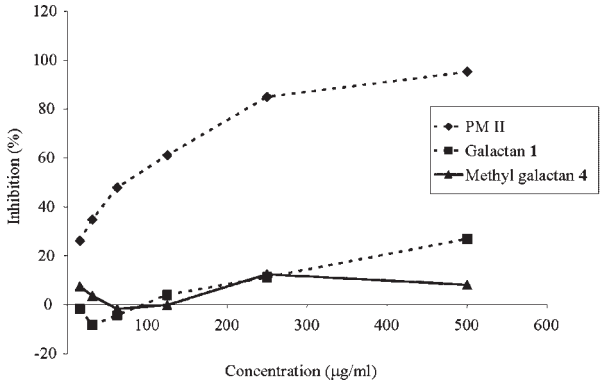

Figure 11. Effect of the galactan **1** and methyl galactan **4** on the complement fixation assay. PM II is the standard polysaccharide.

fraction isolated from *Acanthus ebracteatus*. This result was explained with missing superstructure of other polysaccharides, in particular pectins that accompany the 3-*O*-methyl galactose-rich fractions.

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