Engineered Polysaccharides: α-1,3-Glucan Acetates Showing Upper Critical Solution Temperature in Organic Solvents

Thomas Heinze,* Annett Pfeifer, Andreas Koschella, Douglas Adelman, Laurie Howe, Natnael Behabtu, and Christian Lenges

Acetates of α-1,3-glucan dissolved in *N,N*-dimethyl acetamide/LiCl are prepared by converting the polysaccharide with acetyl chloride, acetic acid anhydride/pyridine, or with acetic acid/*N,N'*-carbonyl diimidazole. Values of the degree of substitution for the acetyl groups (DS_{Ac}) of up to 2.6 are realized. NMR spectroscopic measurements reveal a preferred conversion of the primary hydroxyl group at position 6 followed by positions 2 and 4. Depending on the DS_{Ac}, the samples may be soluble in solvents of different polarity at room temperature or at elevated temperatures showing upper critical solution temperature at DS of about 2.5. This process is found to be reversible.

1. Introduction

Polysaccharides of different origin are considered one of the most important current and future resources for sustainable material development. Today, both cellulose and starch are widely used for a broad range of industrial applications. Typically, polysaccharides and their derivatives are refined from plant based biomass inputs. Recently, engineered polysaccharides with structures controlled by enzymatic polymerization using various C-sources are emerging as potentially valuable renewable resources. For example, the enzymatic polymerization of sucrose is advancing toward commercial reality and represents such a recent example. The engineered poly-saccharide

α-1,3-polyglucose (glucan) obtained by conversion of sucrose with a glycosyl transferase enzyme to form the α-1,3-polyglucose and fructose (**Scheme 1**).[1,2]

This structurally uniform, semi-crystalline polysaccharide is not soluble in water and as expected for a highly associated, hydrogen-bonded polymer, does not show a rubbery phase leading to a defined melting point (decomposition > 330 °C). This polysaccharide is finding use in various structural material applications (e.g., in bio-degradable films or fibers).[1] Moreover, as established chemistries for the functionalization of polysaccharides can be utilized as well for these new structures, chemical functionalization will enhance the spectrum of properties significantly. In this regard, oxidized derivatives,[3] nonionic ethers[4] as well as ionic ethers, and various organic esters have been described so far.[5] In particular esters are of importance as they offer a wide range of structural control due to the variability of the existing carboxylic acids. These novel glucan esters are soluble in organic media and can be shaped into films; also, these glucan esters will allow for direct melt-processing of these materials through various important plastic shaping technologies.[6]

Acetylation is a simple path to functionalize the polysaccharide backbone. For instance, the industrial production of cellulose acetate has started more than 100 years ago.[7] In addition to the industrially applied procedures, numerous other acylation techniques have been developed in the past that comprise the utilization of acid chlorides as reactive compounds or the application of in situ activation techniques as reviewed.[8] β-1,3-Glucan esters were found to form spherical nanoparticles by dialysis of their acetone solution against water.[9] Other methods for the preparation of α-1,3-glucan esters of aliphatic carboxylic acid esters have been reported.[10] However, typically large excess of reagent (7 mol per mol repeating unit) and long reaction time (96 h at 60 °C) were reported. The functionalization pattern is known to influence the properties of the polysaccharide derivatives as well. Moreover, the inherent structure of the polysaccharide may direct substituents kinetically in a certain position or may cause migration within the repeating unit during the course of reaction.[11] Polysaccharide derivatives dissolve in solvents of different polarity depending on the type of substituent and the degree of substitution (DS). However, their solubility may depend on the temperature. Cellulose ethers, for example, may

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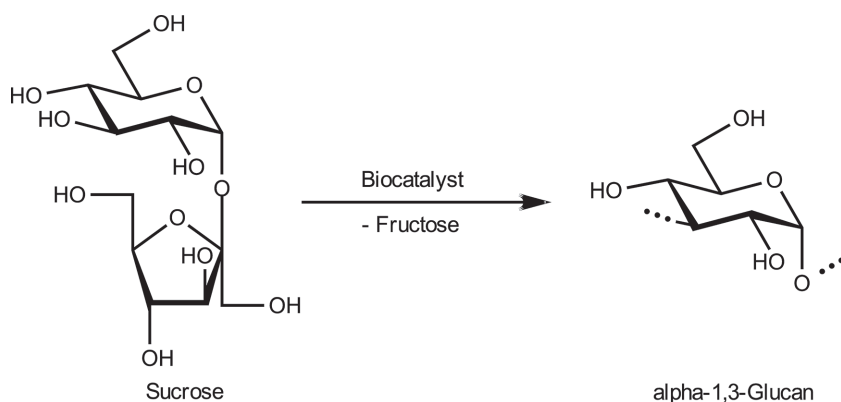E-mail: thomas.heinze@uni-jena.de
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Scheme 1. Bioconversion of sucrose to α-1,3-glucan.

possess a lower critical solution temperature (LCST), that is, the polymer becomes insoluble by heating to a certain temperature forming a precipitate or a gel. This behavior is observed in both aqueous[12,13] and organic solutions of polysaccharide ethers.[14] Polysaccharide derivatives showing upper critical solution temperature (UCST) are scarcely described in the literature. Both LCST and UCST are described for mixtures of cellulose acetate and acetone[15,16] or 2-butanone.[17] Inorganic esters like cellulose nitrate[18] or even cellulose itself were found to exhibit UCST.[19] Moreover, phase transitions have been observed for cellulose derivatives mixed or grafted with other polymers.[20–23] Xanthan gums were found to be a UCST polymer as well.[24] Such materials have found increasing interest as stimuli-responsive polymers for the preparation of smart materials.[25] Polysaccharides are advantageous here due to their inherent biocompatibility and absence of hazardous monomers.

In the present paper, we wish to report on the acetylation of α-1,3-glucan, obtained by enzymatic polymerization of sucrose,[26] and the properties of the resulting ester derivatives in terms of reaction conditions versus DS, functionalization pattern, and the thermo-responsive properties of the dissolved glucan esters.

2. Experimental Section

2.1. Materials

α-1,3-Glucan **1** was kindly provided by DuPont. Degree of polymerization (DP) of 288 (number average, DP_n) and weight average, DP_w of 1343 were measured by SEC of the carbanilated polymer (see Section 3.1) The polymer was dried at 100 °C in vacuum over potassium hydroxide prior to use. *N,N*-Dimethyl acetamide (DMA), acetyl chloride, phenyl isocyanate, pyridine, and propionic anhydride were purchased from Sigma- Aldrich and used as received. Lithium chloride (Sigma-Aldrich) was dried at 150 °C in vacuum over potassium hydroxide.

2.2. Measurements

FTIR spectra were acquired with a Nicolet Avatar DTGS FTIR spectrometer applying the KBr-technique.

NMR-spectra were recorded with Bruker devices operated at 250 and 400 MHz. Samples were dissolved in chloroform-d and measured at room temperature. 16 scans were collected for [1]H- and up to 10240 scans were collected for ^{13}C-NMR spectra. The solvent signal was used as internal reference.

The SEC setup contains a LC-10AD VP pump, columns (PSS SDV pre, PSS SDV lin M) as well as UV–vis- and refractive index detectors. THF with a flow rate of 1 mL min^{-1} was used as eluent.

The sugar composition of the α-1,3-glucan was determined by HPLC after complete depolymerization with $HClO_4$ according to previously published papers.[27,28]

The visual inspection of the thermoreversible gelation was conducted as follows: Glucan acetate (0.03 g) and solvent (3 mL) were homogenized in a sample vial and allowed to rest for 1 h. The vial was then placed in a thermostated aluminium block and equilibrated for 10 min. Dissolution/gelation was visually inspected before the temperature was changed incrementally by 10 K in both directions. A flowing mixture was considered as solution, while no movement of the mixture was considered as gel. The thermal behavior of the polymer solutions was studied using a SETARAM micro DSC-III calorimeter (Caluire, France). About 850 mg of each sample solution, with pure solvent as a reference, were sealed into DSC cells.

2.3. Synthesis

2.3.1. Carbanilation of α-1,3-Glucan (Sample **1a**)

A suspension of α-1,3-glucan **1** (0.5 g) in 15 mL DMA was stirred for 2 h at 120 °C. LiCl (0.9 g) was added after cooling to 80 °C. During cooling to room temperature, the biopolymer dissolved yielding a clear and viscous solution. Pyridine (2 mL, 8 mol/mol AGU) and phenylisocyanate (4.2 mL, 12.5 mol per mol AGU) were added. The reaction mixture was allowed to react for 5 h at 70 °C under stirring. The product was isolated by precipitation into 150 mL methanol, washing with methanol (three times, 100 mL) and drying at 80 °C under vacuum.

Yield: 1.13 g
DS = 2.97 (M of repeating unit = 516.0 g mol^{-1})
Elemental analysis: 61.69 % C, 4.92 % H, 8.07 % N
The sample dissolves in THF.

2.3.2. Acetylation of α-1,3-Glucan (Sample **2i**)

A suspension of α-1,3-glucan **1** (2.0 g) in 60 mL DMA was stirred for 2 h at 120 °C. LiCl (3.0 g) was added after cooling to 80 °C. During cooling to room temperature, the biopolymer dissolved yielding a clear and viscous solution. Acetyl chloride (4.40 mL, 4.84 g, 5 mol per mol AGU) was added. The reaction mixture was allowed to react for 4 h at 80 °C under stirring. The product was isolated by precipitation into 500 mL ethanol,

washing with ethanol (three times, 350 mL) and drying at 80 °C under vacuum.

Yield: 3.66 g

DS_{Ac} 2.48 (determined by ^{1}H-NMR spectroscopy after perpropionylation)

FTIR spectroscopy (KBr, v, cm^{-1}): 3479 v (OH); 2867 v(CH); 1749 v(C=O); 1435 δ(CH$_2$), 1379 δ(CH$_3$); 1226 v(C–O–C$_{ester}$); 1000 v(C–O–C$_{AGU}$).

The sample dissolves in chloroform at room temperature and swells in DMA and DMF.

2.3.3. Perpropionylation of α-1,3-Glucan Acetate 2i (Sample 3i)

A mixture of glucan acetate **2i** (0.5 g) and pyridine (5.0 mL) was heated to 80 °C under stirring. Propionic acid anhydride (5.0 mL) and 50 mg *N,N*-dimethylaminopyridine were added and stirring was continued at 80 °C for 24 h. Due to the gelation of the reaction mixture at room temperature, the hot mixture was poured into 150 mL ethanol. The precipitated polymer was collected, washed three times with 100 mL ethanol, and dried in vacuum at 80 °C.

Yield: 0.5 g

DS_{Ac} 2.48 (determined by ^{1}H-NMR spectroscopy after perpropionylation)

^{13}C-NMR spectroscopy (CDCl$_3$, 100.63 MHz, δ, ppm): 8.66 CH$_3$ (propionate); 20.73 CH$_3$ (acetate); 61.39 C-6$_s$; 67.78, 70.11, 71.81, 77.53 C-2, 3, 4, 5; 94.85 C-1′, 169.33, 170.19, 170.52 C=O (acetate).

The sample dissolves in chloroform.

3. Results and Discussion

3.1. Properties of the α-1,3-Glucan

A solution of a molecularly dispersed polymer is a prerequisite for conducting effective SEC analysis. The reaction of α-1,3-glucan with phenylisocyanate in presence of pyridine was carried out in order to facilitate the preparation of a readily soluble polymer system. The resulting glucan phenylcarbamate is almost fully functionalized (DS 2.97) and dissolves in *N,N*-dimethyl acetamide (DMA), tetrahydrofuran (THF), and chloroform. It is insoluble in dimethyl sulfoxide (DMSO) and usually reflects the molecular weight distribution of the starting polymer. SEC analysis of this glucan phenylcarbamate revealed a molar mass distribution with a main component (peak maximum ca. 300 000 g mol^{-1}) and a small shoulder at a peak maximum of ca. 20 000 g mol^{-1}, which results in M_n 104 970 g mol^{-1} (number average degree of polymerization, DP$_n$ 203), M_w 685 960 g mol^{-1} (weight average degree of polymerization, DP$_w$ 1329), and Đ 6.53 (**Figure 1**). Separate data processing of main- and side components has changed the picture a bit: M_n 148 430 g mol^{-1} (DP$_n$ 288), M_w 693 140 g mol^{-1} (DP$_w$ 1343), and Đ 4.67. The smaller fraction has a M_n of 3783 (DP$_n$ 7) and a M_w of 4002 g mol^{-1} (DP$_w$ 8) with a Đ of 1.04. For better comparison, the SEC curves are normalized to the non-functionalized repeating unit in order to exclude the influence of the substituent on the molar mass of the repeating unit.
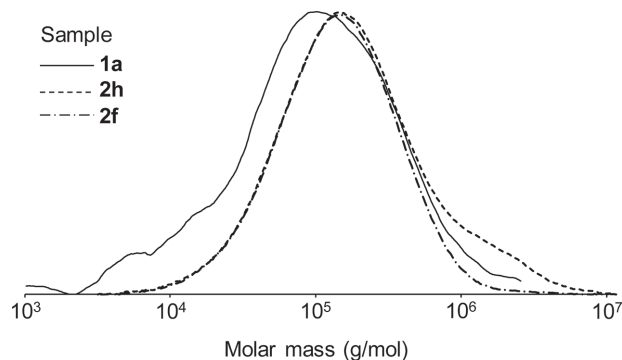

Figure 1. SEC curves of α-1,3-glucan phenylcarbamate **1a** and α-1,3-glucan acetates **2h** and **2f**. The molar masses are normalized to the non-functionalized polymer backbone.

The α-1,3-glucan **1** exhibits a solubility that is similar to β-1,4-linked cellulose (**Table 1**), except the already noted solubility in sodium hydroxide.[29]

NMR spectra (acquired in DMSO-d_6/LiCl) revealed an essentially exclusive structural assignment for the α-1,3-linkage supporting the selectivity of the enzymatic polymerization process (**Figure 2**). The peak assignment was carried out by means of 2D-techniques (^{1}H/^{1}H-COSY, HSQC/DEPT). The peaks in the ^{1}H- and ^{13}C-NMR spectrum were assigned: 5.07/100.0 ppm (H1/C1), 3.35/71.47 ppm (H2/C2), 3.63/82.81 ppm (H3/C3), 3.30/70.15 ppm (H4/C4), 3.81/72.60 ppm (H5/C5), and 3.57; 3.49/60.94 ppm (H6$_a$ and H6$_b$/C6). The protons of the hydroxyl groups were detected at 4.64 ppm (C2-OH), 5.19 ppm (C4-OH), and 4.47 ppm (C6-OH). The 1,3-glycosidic linkage becomes obvious from the deep-field shifted signal of C3. The peak assignment is in agreement with a previously published work.[2]

3.2. Acetylation and Structure Characterization of the α-1,3-Glucan

Acetylation was carried out by homogeneous reaction of the biopolymer in DMA/LiCl with acetyl chloride for 4 h at

Table 1. Solubility of α-1,3-glucan **1** in different solvents.

Solvent[a]	Solubility at		Addition of salt
	r.t.[b]	100 °C	
Water	–	–	–
5 % NaOH, water	+	+	–
DMA	–	–	+/LiCl
DMA	–	–	+/NEt$_3$OctCl[d]
DMSO	–	–	+/LiCl
DMF	–	–	–/LiCl
Imidazole	n.d.[c]	–	–[e]
BMIMCl	n.d.[c]	+	–[f]

[a] *N,N*-Dimethyl acetamide (DMA), dimethyl sulfoxide (DMSO), *N,N*-dimethyl formamide (DMF), 1-butyl-3-methylimidazolium chloride (BMIMCl); [b] Room temperature (rt); [c] Not determined (n.d.); [d] Triethyloctylammonium chloride (NEt$_3$OctCl); [e] Melt; [f] Tolerates up to 50 % DMF as diluent.
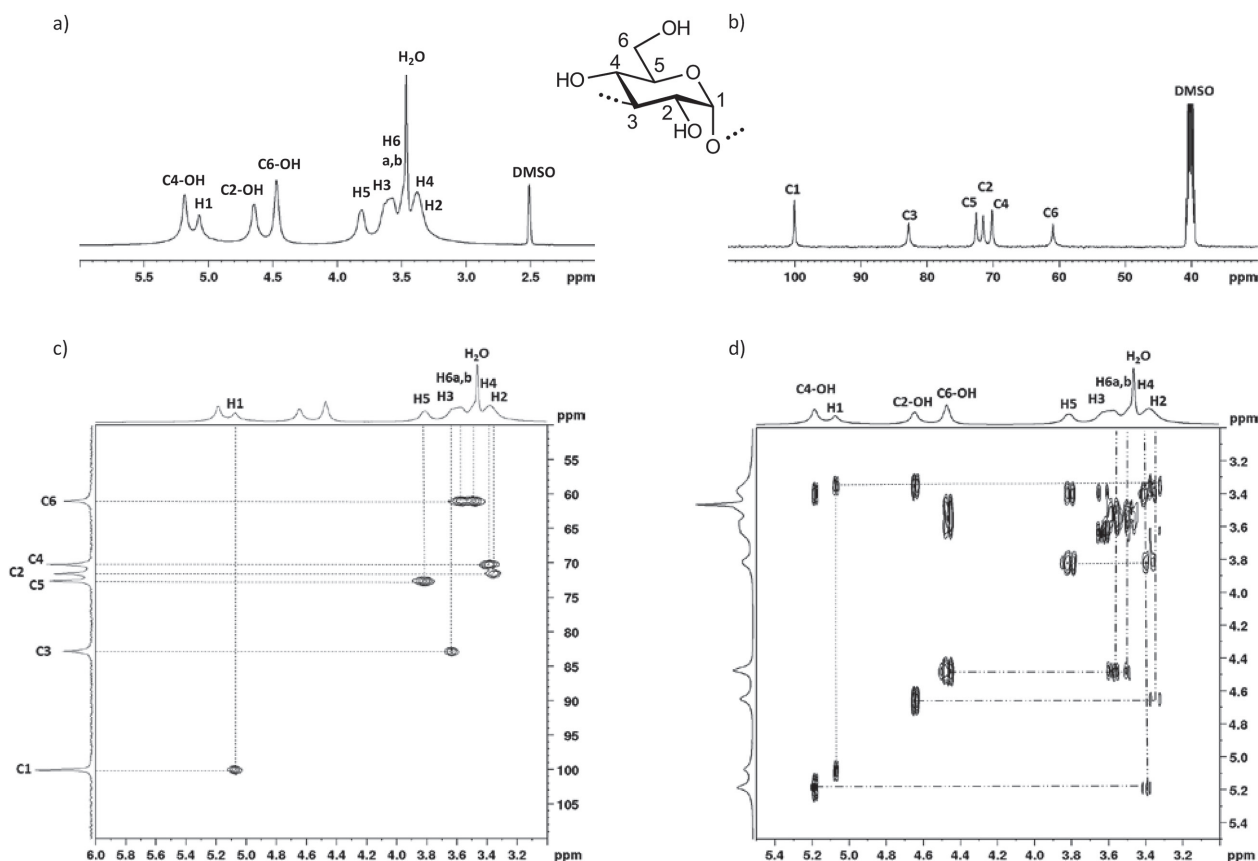
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Figure 2. a) ^{1}H-, b) ^{13}C-, c) HSQC-DEPT-, and d) ^{1}H/^{1}H-COSY NMR spectra of α-1,3-glucan **1** recorded in dimethyl sulfoxide (DMSO)-d_6/LiCl at 60 °C.

80 °C followed by precipitation in ethanol, washing, and drying (**Scheme 2**). The molar ratio was chosen to achieve different DS values (**Table 2**). The DS of acetyl groups (DS$_{Ac}$) was determined by ^{1}H-NMR spectroscopy of the fully propionylated polymer or without additional functionalization in the presence of trifluoroacetic acid. Acetyl chloride, a highly reactive reagent, was used because it allows to get products with a controlled and even with high DS values applying stoichiometric amounts. The efficiency of acetyl chloride as acetylation reagent is well known from the literature.[30] Applying 0.8 mol acetyl

chloride per mole repeating unit, a DS$_{Ac}$ of 0.44 was achieved (sample **2a**), which corresponds to a conversion of 55 % of the reagent. Further increase of the molar ratio repeating unit to reagent led to an expected increase of the DS$_{Ac}$ with reagent efficacies between 48 and 94 %. Although, acetyl chloride is highly reactive, a maximum DS$_{Ac}$ of 2.35 could be realized after 4 h reaction time (sample **2h**). Further increase of the molar ratio anhydroglucose unit:acetyl chloride from 1:3 to 1:5 led to a slight increase of the DS$_{Ac}$ to values up to 2.6 (sample **2j**) but a complete acetylation was not achieved under these conditions.
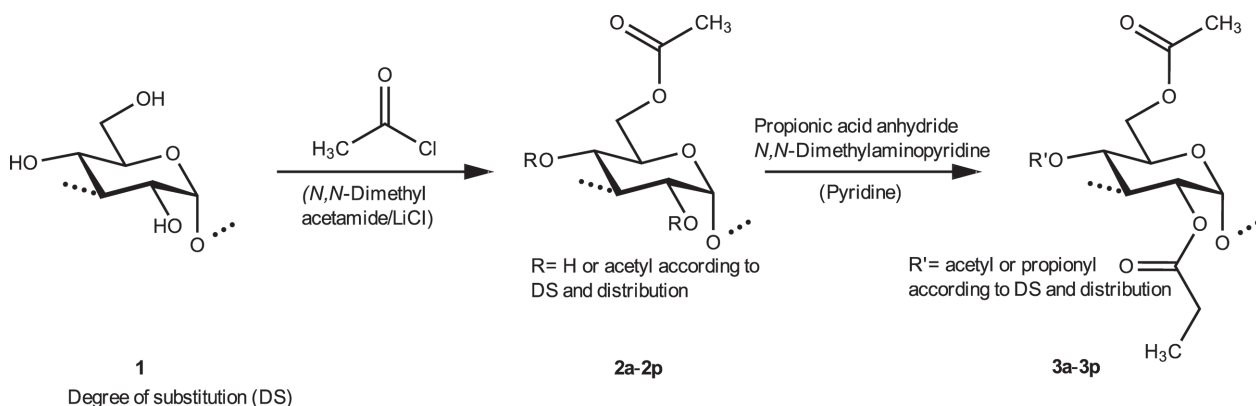
Scheme 2. Conversion of α-1,3-glucan **1** under homogeneous reaction conditions with acetyl chloride followed by perpropionylation for analytical purposes.

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Table 2. Conditions for and results of the acetylation of α-1,3-glucan **1** with acetyl chloride in N,N-dimethyl acetamide/LiCl solution for 4 h at 80 °C.

Molar ratio[a]	Sample	DS_{Ac}[b]	Solubility at r.t.[c]						Solubility at 60 °C					
			DMA	DMSO	DMF	AcCN	THF	Chl.	DMA	DMSO	DMF	AcCN	THF	Chl.
1:0.8	**2a**	0.44	−	−	n.d.	−	−	−	−	+	n.d.	−	−	−
1:1.3	**2b**	1.22	+	−	n.d.	−	−	−	n.d.	+	n.d.	−	−	−
1:1.8	**2c**	1.18	+	−	n.d.	−	−	−	n.d.	+	n.d.	−	sw	−
1:2.3	**2d**	1.81	+	sw	n.d.	−	sw	+	n.d.	sw	n.d.	+	+	n.d.
1:2.3	**2e**[d]	1.80[i]	+	sw	n.d.	sw	+	+	n.d.	+	n.d.	+	n.d.	n.d.
1:2.3	**2f**[e]	1.92[i]	+	+	n.d.	sw	+	+	n.d.	n.d.	n.d.	+	n.d.	n.d.
	2g	1.92	sw	sw	sw	sw	sw	sw	+	+	+	+	+	+
1:3.0	**2h**	2.35	+	+	n.d.	+	+	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
1:5.0	**2i**	2.48	sw	sw	n.d.	n.d.	−	+	+	sw	n.d.	n.d.	+	n.d.
1:5.0	**2j**	2.61	sw	sw	sw	sw	sw	+	+	sw	+	+	+	n.d.
1:5.0	**2k**[d]	2.12[i]	+	+	n.d.	n.d.	+	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
1:5.0	**2l**[e]	2.56[i]	+	sw	n.d.	sw	+	+	n.d.	+	n.d.	+	n.d.	n.d.
1:5.0	**2m**[f]	2.70	sw	sw	n.d.	sw	sw	+	+	sw	n.d.	+	+	n.d.
1:4.0	**2n**[g]	1.35[i]	sw	sw	n.d.	n.d.	−	−	+	+	n.d.	−	+	n.d.
1:4.0	**2o**[h]	2.18	+	+	n.d.	+	+	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	2p[j]	2.18	sw	sw	sw	sw	sw	sw	+	+	+	+	+	+

[a]Molar ratio anhydroglucose unit (AGU): acetyl chloride; [b]Degree of substitution of acetyl groups (DS_{Ac}) determined by means of ^{1}H-NMR spectroscopy of the per-propionylated samples; [c]Room temperature (r.t.), acetonitrile (AcCN), N,N-dimethyl acetamide (DMA), N,N-dimethyl formamide (DMF), dimethyl sulfoxide (DMSO), tetrahydrofuran (THF), chloroform (Chl.) soluble (+), insoluble (−), swells (sw; volume of the solid sample material increases significantly without forming a homogeneous mixture); [d]1 h, 10 °C and 16 h, room temperature; [e]1 h, 10 °C; 16 h, room temperature; and 1 h, 80 °C; [f]Reaction time 16 h; [g]Acetic acid/N,N′-carbonyldiimidazole (20 h, 70 °C, 4 mol per mol AGU) instead of acetyl chloride; [h]Acetic anhydride/pyridine (48 h, 60 °C) instead of acetyl chloride; [i]DS_{Ac} was determined by ^{1}H-NMR spectroscopy of the glucan acetate in DMSO-d_6 with addition of trifluoroacetic acid; [j]Obtained by peracetylation of **2o**.

Change of reaction time and temperature did not yield samples with higher DS_{Ac}, for example, 16 h conversion (DS_{Ac} 2.70, sample **2m**) or conversion for 1 h at −10 °C followed by 16 h at room temperature and 1 h at 80 °C (DS_{Ac} 2.56, sample **2l**). Similar results were found applying other acylation agents. A reaction with 4 mol acetic acid and N,N′-carbonyl-diimidazole (20 h, 70 °C) was less effective (DS_{Ac} 1.35, sample **2n**) and a 48 h conversion with acetic anhydride and pyridine lead to a DS_{Ac} of 2.18 (sample **2o**). On the contrary to results using large reagent excess and long reaction time,[10] a fully acetylated α-1,3-glucan could not be synthesized in a single reaction step under moderate conditions investigated here.

The DS_{Ac} is usually determined by means of ^{1}H-NMR spectroscopy of the perpropionylated polymer. Peracylation is necessary to provide a polymer without remaining hydroxyl groups. These polymers are soluble in chloroform (no signals in the peak range of interest) and do not interact by means of hydrogen bonds. An intriguing observation was that perpropi-onylation of samples with $DS_{Ac} > 2.2$ could not be achieved, which is in complete contrast to other polysaccharide ester systems like dextran,[31] cellulose,[32] and starch.[33] It is assumed that the full accessibility of the hydroxyl groups is not given by a conformation of the glucan acetate in solution. In this case, ^{1}H-NMR spectra were recorded in DMSO-d_6 solution with addition of trifluoroacetic acid in order to shift the signals of hydroxyl protons which may interfere with signals used for the DS calculation. The signals, whose intensities depend on the particular DS, could be assigned: 8.78 ppm (CH$_3$-propionate),

20.85 ppm (CH$_3$-acetate), 61.41 ppm (C-6s), 66.82–71.65 ppm (C-2-C-5), 94.80 ppm (C-1), 93.48 (C-1′), 169.43-170.69 ppm (C=O acetate), and 172.84–173.74 ppm (C=O propionate). The resolution of the NMR spectra is not as good as the resolution of spectra taken from perpropionylated cellulose acetates. In-depth analyses by two-dimensional NMR spectroscopy, in particular the assignment of the carbonyl peaks could not be carried out. Therefore, the carbonyl signals have been assigned according to the literature.[11] **Figure 3** presents the ranges of methyl- and carbonyl resonances in the ^{13}C-NMR spectra of perpropionylated α-1,3-glucan acetates having DS_{Ac} values in the range from 0.44 to 2.48. The carbonyl signals exhibit significant changes that depend on the DS_{Ac} of the sample. Starting with DS_{Ac} 0.44 (sample **3a**), only one signal for the C=O (acetate) appeared together with a set of three signals for C=O (propionate). It is known that the chemical shift of the carbonyl signals depends on the particular position within the repeating unit. Owing to the highest reactivity of primary hydroxyl groups under homogeneous reaction conditions, pre-ferred and partial acetylation of position 6 must be concluded. With increasing functionalization, a signal at 169.3 ppm together with a shoulder at 170.17 ppm started to appear in the ^{13}C-NMR spectrum of sample **3b** (DS_{Ac} 1.22). Obviously, acety-lation of the secondary OH functions occurs. In this regard, the shape of the peaks of C=O (propionate) changed accordingly. Three distinct peaks could be observed at DS_{Ac} 0.44, while only two resonances were detected at DS_{Ac} 1.22 (sample **3b**). Here, the signal at 173.9 ppm is reduced to a shoulder. The intensity
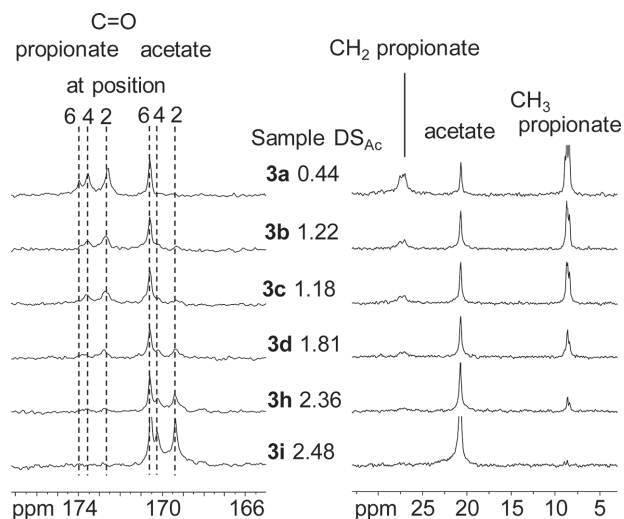
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Figure 3. Carbonyl (left) and methyl (right)-region of ^{13}C-NMR spectra of perpropionylated α-1,3-glucan acetates of different degree of substitution of acetyl groups (DS$_{Ac}$) recorded in CDCl$_3$.

Figure 4. Temperature-dependent appearance of glucan acetate **2j** (DS$_{Ac}$ 2.61, 2 % w/v) in different solvents according to the symbols. The results of one solvent are connected by the lines for better readability.

of the C=O (propionate) decreases as the intensity of the C=O (acetate) signals increases with further increase of the DS$_{Ac}$. A reactivity order of position 6 > 2 ≫ 4 can be concluded based on these observations.

3.3. Characterization of Macromolecular and Thermal Properties of the α-1,3-Glucan Esters

Selected glucan acetate samples have been subjected to SEC analysis (Figure 1). Molar masses of sample **2h** were determined: M_n 149410 g mol^{-1} (DP$_n$ 573), M_w 601170 g mol^{-1} (DP$_w$ 2328), and Đ 4.02. The apparent molar masses are larger compared with the data of starting material **1**. Therefore, it may be possible that some additional aggregation is still present in the acetylated samples, even at DS$_{Ac}$ 2.35. There is no significant difference between the glucan acetates in terms of number average molar mass. Comparing samples **2h** and **2f** M_n of **2f** (129190 g mol^{-1}, DP$_n$ 532) are almost identical.

The solubility of these glucan esters exhibited an unexpected behavior. As it is summarized in Table 2, these are soluble or swellable in DMA starting at DS$_{Ac}$ 1.22 without clear correlation with the DS$_{Ac}$. Interestingly, the swollen polymers (**2g, 2i, 2j, 2m, 2n, 2p**) formed homogeneous solutions upon heating the mixtures to 60 °C. In case of DMSO, samples with DS$_{Ac}$ up to 1.18 do not dissolve at room temperature but dissolve at 60 °C. Starting at DS$_{Ac}$ 1.81, the samples swell at room temperature and dissolve upon heating. A clear trend has been observed in the solvents DMF and acetonitrile, where the samples swell at room temperature and
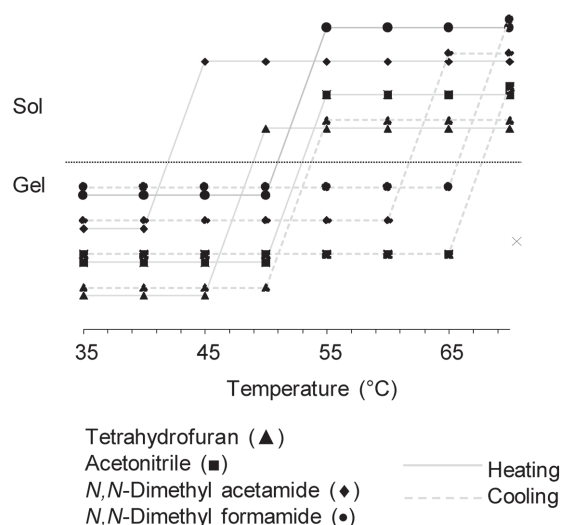
dissolve at 60 °C. Expectedly, a higher DS$_{Ac}$ of 1.81 is required for solubility in less polar solvents like THF and chloroform. The samples form almost clear solutions at room temperature, which became completely clear at 60 °C.

The temperature-dependent solubility of sample **2j** (DS$_{Ac}$ 2.61) was checked manually by visual inspection of samples mixed with different solvents at different temperatures (**Figure 4**). It can be seen that the solubilization/gelation depends on the solvent and exhibits a distinct hysteresis. The lowest solubilization temperature has been observed in DMA, followed by THF. There was no significant difference found between DMF and acetonitrile. The gels are slightly turbid, while the solutions are optically clear. By examining samples with DS$_{Ac}$ below 2.5 no UCST behavior could be observed.
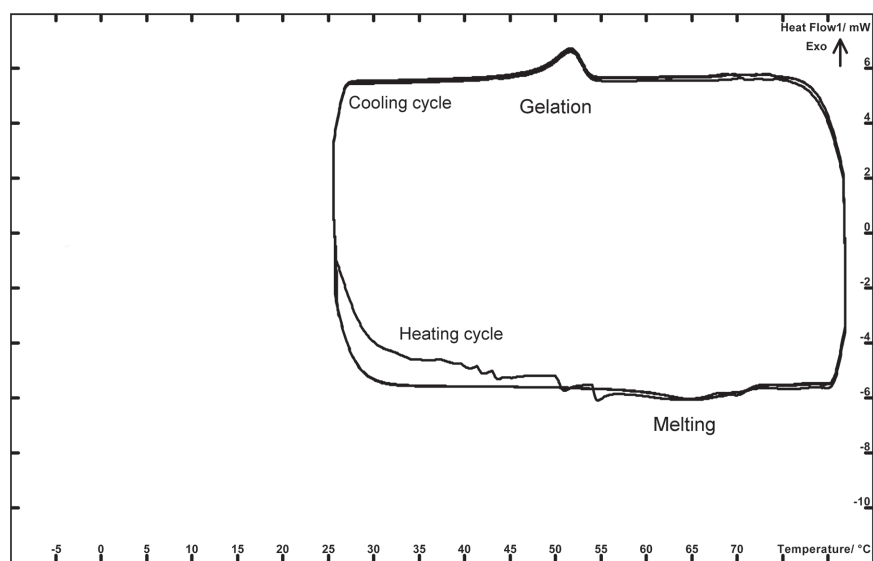

Figure 5. micro-DSC plot of α-1,3-glucan acetate **2j** (DS$_{Ac}$ 2.61, 2 % w/v) in acetonitrile solution.

Table 3. Thermal parameters of sample **2j** determined by micro-DSC in acetonitrile solution.

	Run 1	Run 2
Heating		
Onset temperature [°C]	54.64	54.68
Peak temperature [°C]	52.94	52.95
Enthalpy [j g^{-1}]	−0.4039	−0.5088
Cooling		
Onset temperature [°C]	57.39	61.01
Peak temperature [°C]	64.71	64.88
Enthalpy [j g^{-1}]	0.5086	0.4712

Samples of comparably high DS_{Ac} were found to exhibit UCST behavior. Therefore, sample **2j** (DS_{Ac} 2.61) has been exemplarily studied by means of microcalorimetry. **Figure 5** shows the result of three heating/cooling cycles. The melting range during heating is identical in every heating cycle. A closer look at the thermal data (**Table 3**) showed that both, peak- (52.9 °C) and onset temperature (54.6 °C) are identical in the heating cycle of the last two runs. The difference in enthalpy is within the error range of the method. However, the gelation is obviously a thermal event occurring at longer timescale yielding a broader peak. This caused slight deviations in the onset temperatures (1st run: 57.30 °C, 2nd run: 61.01 °C). However, the peak temperatures were almost identical (ca. 64.8 °C). The values of the enthalpy are in accordance with the enthalpy of the melting process.

4. Conclusions

To conclude, enzymatic polymerization allows access to novel engineered polysaccharides such as α-1,3-glucan **1**, which could be successfully acetylated under homogeneous reaction conditions in DMA/LiCl yielding product with DS_{Ac} of up to 2.61. Complete acetylation was not achieved in this study, which is in contrast to other typical polysaccharides such as cellulose using similar reaction conditions. The glucan acetates synthesized possess a DS-dependent solubility and show the interesting effect of UCST at DS values around 2.5. The working hypothesis that the conformation of the polymer in solution may limit the accessibility of the hydroxyl groups must be investigated in further studies. Due to the fact that swelling is observed at low temperature followed by dissolution upon heating and gelation upon cooling, the glucan esters presented in this work point toward a novel strategy to design new thermoresponsive polymers. Through on-purpose design of novel engineered polysaccharides in combination with established derivatization methodology, inherently renewable and biodegradable thermoresponsive polymer systems can be envisioned. Typical applications of these highly biocompatible polymers are emerging in the areas of organogelators, rheology control additives, drug delivery materials, carrier or sensor material providing extended use and application ranges compared with established technologies.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Conflict of Interest

The authors declare no conflict of interest.

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