Synthesis and characterization of regioselectively substituted curdlan hetero esters via an unexpected acyl migration

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Abstract

Regioselectively substituted curdlan esters were synthesized by protecting the C6 primary hydroxyl group with a triphenylmethyl group followed by the acylation of the secondary hydroxyl groups at C2 and C4. The subsequent detritylation of C6 trityl group under acidic conditions revealed an unexpected acyl migration from C4 to C6. This unique acyl migration in curdlan was first observed, which haven't been reported in other polysaccharides such as cellulose. The rate of this migration was found to be dependent on the length of the acyl group, leading to the proposal of a new mechanism for this transformation. Based on these results, we synthesized 2,6-di-*O*-acetyl-4-*O*-propionyl-curdlan, which was fully characterized by ¹H NMR, ¹³C NMR, COSY, HSQC and HMBC analyses. Thermogravimetric analysis and differential scanning calorimetry measurements revealed that the regioselective esterification to curdlan promoted its crystallization compared with randomly mixed ester derivatives.

Keywords: curdlan, regioselectively, hetero ester, acyl migration

1. Introduction

Curdlan (CD) is linear homopolysaccharide consisting of β-(1→3)-D-glucose units that is produced by microorganisms such as *Alcaligenes faecalis* (Misaki & Saito, 1968; Harada, Harada, 1977; Harada, Terasaki & Harada, 1993; McIntosh, Stone & Stanisich, 2005;

Paul, Morin & Monsan, 1986; Sutherland, 1994). Curdlan is mainly used as a food additive because of its thermogelable characteristics. Besides, curdlan and its derivatives have also been reported to show considerable potential in medical applications because of their antitumor ability (Yoshida et al., 1990, Borjihan, Zhong, Baigude, Nakashima & Uryu, 2003; Hrada et al., 2001). Based on their high molecular weight, nontoxic nature and biodegradable characteristics, curdlan and its derivatives are also being studied as environmentally friendly alternatives to oil-based plastics.

Curdlan contains a large number of inter- and intramolecular hydrogen bonding interaction between the many hydroxyl groups on the glucose units making up its complex polymeric structure. Curdlan therefore exhibits poor solubility towards organic solvents, as well as being non-thermoplastic, as like other polysaccharides. Esterification represents an efficient strategy for reducing the hydrogen bonding capability of hydrogen-rich compounds, and can be used as to improve the thermo- and mechanical properties of polysaccharides. It has been reported that the thermal stability and solubility of curdlan were improved by esterification and that resulting curdlan esters showed thermoplastic characteristics, making them potential alternatives to plastics. The thermal and mechanical properties of curdlan, as well as its crystallinity, can be controlled by varying the length of its ester chains (Marubayashi, Yukinaka, Enomoto-Rogers, Takemura & Iwata, 2014).

The monosaccharides units making up polysaccharide chains contain more than one

hydroxyl group. Substituted functional group on each hydroxyl position has different contribution to the structure and properties of polysaccharide derivatives. Several studies have recently been reported with aim of developing a deeper understanding of the relationship between the structure and properties of polysaccharide derivatives via synthesis of regioselectively substituted polysaccharide system. Notably, the regioselective synthesis of substituted cellulose derivatives made it possible to control the crystallinity and melting behaviors of a series of cellulose ester (Iwata, Azuma, Okamura, Muramoto & Chun, 1992; Iwata, Okamura, Azuma, Chanzy & Tanaka, 1994; Iwata, Okamura, Azuma & Tanaka, 1996a; Iwata, Okamura, Azuma & Tanaka, 1996b; Iwata, Doi & Azuma, 1997; Iwata, Fukushima, Okamura & Azuma, 1997). Furthermore, the formation of inter- and intramolecular hydrogen bonding interaction was clarified in these cellulose derivatives (Kondo, 1994). The regioselective substitution of polysaccharides may also result in unconventional properties, as exemplified by 6-amino-6-deoxy curdlan sulfate, which exhibited anti-HIV activity (Borjihan et al., 2003). Furthermore, curdlan bearing C6-branched rhamnosyl or arabinofuranosyl units have been reported to exhibit cancerostatic activity (Demleitner, Kraus & Franz, 1992), whereas 2,3-di-*O*-cellulose ethers have shown unconventional phase separation behaviors. However, regioselective synthesis of substituted polysaccharide derivatives remains a challenging area of research. Furthermore, bulky protecting groups such as trityl (Helferich, & Koester, 1924; Hearon, Hiatt & Fordyce, 1943;

Honeyman, 1947; Hall & Horne, 1973) and silyl (Kamitakahara, 2008; Fenn, Pohl & Heinze, 2009) groups are routinely used in this area to achieve the regioselective synthesis of substituted polysaccharide derivatives based on subtle differences in the reactivity of different hydroxyl and protecting groups. Trityl protection has been used to achieve the synthesis of C6-branched curdlan derivatives by completely protecting the primary hydroxyl groups of the monosaccharide units as trityl ether (Demleitner et al., 1992). Regioselective bromination has also been reported as an efficient method for the regioselective synthesis of substituted 6-*O*-amino-6-*O*-deoxyl curdlan esters (Zhang & Edgar, 2014).

The aim of this study was to develop a deeper understanding of the relationship between the substitution position of the ester group and the properties of resulting curdlan esters. We have regioselectively synthesized a series of curdlan esters using a trityl group to protect the primary C6 hydroxyl group of glucose monomers. We have also evaluated the thermal properties of the resulting curdlan esters. Notably, we observed an unexpected acyl migration during the detritylation of the trityl curdlan esters and have proposed a plausible mechanism to account for this transformation.

2. Materials and methods

2.1. Materials

Curdlan and lithium chloride were purchased from WAKO (Tokyo, Japan) and vacuum

dried before being used. Pyridine, trityl chloride, acetic anhydride, propionic anhydride, propionyl chloride, butyryl chloride, valeryl chloride, heptanoyl chloride, octanoyl chloride, decanoyl chloride, acetic acid, propionic acid, butyric acid, were purchased from WAKO (Tokyo, Japan). Dimethylformamide (DMF) was purchased from KANTO CHEMICAL INDUSTRY CO. INC (Tokyo, Japan).

2.2 Synthesis of 6-*O*-trityl-curdlan (CD6Tr)

The synthesis of 6-*O*-trityl-curdlan was following the tritylation method of cellulose (Iwata et al., 1992).

Predried curdlan (1.00 g) and lithium chloride (1.75 g) were dissolved in DMF (40 mL) under stirring at 70°C, and the resulting solution was treated with pyridine (10 mL) and trityl chloride (3.44 g). The solution mixture was heated at 70°C for 3 days under stirring. The mixture was poured into ethanol (2 L), resulting in the precipitation of the product. This ethanolic solution was stirred for 1 day, then filtered to precipitate, which was washed with ethanol and dried under vacuum at room temperature with phosphorus pentoxide as dehydrating agent to give pure 6-*O*-trityl-curdlan as a white powder with a yield of 2.42 g (97%).

2.3. Synthesis of 2,4-di-*O*-acetyl-6-*O*-trityl-curdlan (CD24Ac6Tr)

Acetic anhydride (2.5 mL) was added to a stirred suspension of CD6Tr (500 mg) in pyridine (10 mL) under stirring at 50°C, and the resulting mixture was heated at same temperature

over 1 night. The mixture was poured into ethanol (500 mL), resulting in the precipitation of the product. This ethanolic suspension was stirred for 1 day at room temperature and then filtered to give the precipitate, which was washed with ethanol and dried under vacuum at room temperature with phosphorus pentoxide as dehydrating agent to give pure 2,4-di-*O*-acetyl-6-*O*-trityl-curdlan as a yellow solid with a yield of 600 mg (99%).

CD24Ac6Tr: ^1H NMR (CDCl_3 with TMS, 500 MHz): δ_{H} : 1.55–2.09 (Ac- CH_3), 3.14 (ring proton-H6), 3.25 (ring proton-H6'), 3.46 (ring proton-H5), 3.74 (ring proton-H3), 4.34 (ring proton-H1), 4.76 (ring proton-H2, H4), 7.22–7.38 (trityl group). ^{13}C NMR (CDCl_3 , 500 MHz): δ_{C} : 21.10–21.27 (Ac- CH_3), 63.56 (ring carbon-C6), 68.98 (ring carbon-C4), 73.38 (ring carbon-C2), 74.44 (ring carbon-C5), 78.16 (ring carbon-C3), 100.81 (ring carbon-C1), 87.20, 127.61–129.09, 143.77–147.21 (trityl group), 169.11–169.46 (Ac-C=O).

2.4. Synthesis of 2,6-di-*O*-acetyl-curdlan (CD26Ac)

The detritylation of 2,4-di-*O*-acetyl-6-*O*-trityl-curdlan was following the detritylation method of tritylated-cellulose ester (Iwata et al., 1992).

CD24Ac6Tr (500 mg) was dissolved in chloroform (20 mL) under stirring at room temperature, and the resulting solution was treated with 30% hydrogen bromide (1 mL). The mixture was then vigorously stirred for 3 mins, resulting in the formation of a precipitate, which was collected and added to a solution of sodium hydrogen carbonate. This mixture was then stirred for 1 day to allow for the neutralization of the precipitate. The precipitate was

then collected by filtration and then re-suspended in water for another day. The product was collected by filtration and dried under vacuum at room temperature with phosphorus pentoxide as dehydrating agent to give pure 2,6-di-*O*-acetyl-curdlan as a light yellow solid with a yield of 164 mg (65%).

CD26Ac: ¹H NMR (pyridine-d₅, 500 MHz): δ_H: 1.93 (C6-Ac-CH₃), 2.45 (C2-Ac-CH₃), 3.79 (ring proton-H₄), 3.97 (ring proton-H₅), 4.27 (ring proton-H₃), 4.54 (ring proton-H₆), 4.70 (ring proton-H_{6'}), 5.07 (ring proton-H₁), 5.33 (ring proton-H₂). ¹³C NMR (pyridine-d₅, 500 MHz): δ_C: 20.91-21.56 (Ac-CH₃), 63.97 (ring carbon-C₆), 69.59 (ring carbon-C₄), 73.30 (ring carbon-C₂), 74.80 (ring carbon-C₅), 83.79 (ring carbon-C₃), 101.62 (ring carbon-C₁), 170.20 (C2Ac-C=O), 170.95 (C6Ac-C=O).

2.5. Synthesis of 2,6-di-*O*-acetyl-4-*O*-propionyl-curdlan (CD26Ac4Pr).

Propionyl chloride (10 e.q) was added to a mixture of CD26Ac (50 mg) in pyridine (2 mL) at 50°C, and the resulting mixture was stirred at the same temperature for 3 hours. The mixture was poured into ethanol (100 mL) with stirring to give a precipitate, which was stirred for 1 day. The precipitate was collected by filtration and re-dissolved in chloroform before being precipitated from a 1:1 (v/v) mixture of ethanol and H₂O. The precipitate was collected by filtration and washed with H₂O before being dried under vacuum condition at 105°C to give pure 2,4-di-*O*-acetyl-6-*O*-propionyl-curdlan as a yellow solid with a yield of 44 mg (72%).

154 CD26Ac4Pr: ^1H NMR (CDCl_3 with TMS, 500 MHz): δ_{H} : 1.10 (Pr-CH₃), 2.06 (C6-Ac-CH₃),
155 2.12 (C2-Ac-CH₃), 2.27 (C4-Pr-CH₂), 3.63 (ring proton-H5), 3.74 (ring proton-H3), 4.01
156 (ring proton-H6), 4.29 (ring proton-H6'), 4.37 (ring proton-H1), 4.81 (ring proton-H2), 4.90
157 (ring proton-H4). ^{13}C NMR (CDCl_3 , 500 MHz): δ_{C} : 9.21 (Pr-CH₃), 21.01-21.23 (Ac-CH₃),
158 27.39 (Pr-CH₂), 62.41 (ring carbon-C6), 68.22 (ring carbon-C4), 72.35 (ring carbon-C5),
159 73.12 (ring carbon-C2), 78.77 (ring carbon-C3), 101.06 (ring carbon-C1), 169.21
160 (C2Ac-C=O), 170.89 (C6Ac-C=O), 172.84 (C4Pr-C=O).

161 **2.6. Synthesis of 2,4-di-*O*-acyl-6-*O*-trityl-curdlan (CD24Acyl6Tr)**

162 Propionic anhydride (2.5 mL) or acid chloride (butyryl chloride, valeryl chloride, heptanoyl
163 chloride, octanoyl chloride, decanoyl chloride 10 e.q) was added to a stirred suspension of
164 CD6Tr (500 mg) in pyridine (10 mL) under stirring at 50°C, and the resulting mixture was
165 stirred at the same temperature for a specified reaction time (propionylation for 4 days,
166 butylation for 5 h, valerylation to decanoylation for 1 day). The reaction mixture was poured
167 into ethanol (500 mL) to give a precipitation, which was stirred for 1 day. The precipitate was
168 then collected by filtration and washed with ethanol before being dried under vacuum
169 condition at room temperature to give pure 2,4-di-*O*-acyl-6-*O*-trityl-curdlan bearing an acyl
170 chain length from propionate to decanoate.

171 **2.7. Detritylation of CD24Acyl6Tr**

172 A stirred solution of CD24Acyl6Tr (500 mg) in chloroform (20 mL) was treated with 30%

hydrogen bromide (1 mL) under vigorous stirring, and the resulting mixture was stirred for a specific reaction time (CD24Pr6Tr for 3 mins, CD25Bu6Tr for 5 mins, CD24Va6Tr for 6 min, CD24He6Tr and CD24Oc6Tr for 10 mins, CD24De6Tr for 12 mins). CD24Pr6Tr and CD26Bu6Tr formed yellow precipitates, which were collected by filtration and suspended in a solution of sodium hydrogen carbonate. The mixtures were stirred for 1 day to allow to achieve neutralization of the product. All of the other reactions were neutralized by pouring the reaction mixture into sodium hydrogen carbonate solution, resulting in the formation of a biphasic mixture. The chloroform layer was collected and evaporated to dryness. To give a residue, which was suspended in aqueous sodium hydrogen carbonate with stirring for 1 day. In all cases, this mixture was then filtered to give a solid, which was re-suspended in water with stirring for 1 day before being collected by filtration and dried under vacuum conditions at room temperature to give the pure product.

2.8. Determination of the migration ratio detritylated CD24Acyl6Tr

Acetic anhydride (0.3 mL) was added to a stirred mixture of detritylated CD24Acyl6Tr (50 mg) in pyridine (2 mL) at 50°C, and the resulting mixture was stirred at the same temperature overnight. The mixture was poured in ethanol or H₂O (100 mL) to give a precipitate, which was stirred at room temperature for 1 day. The precipitate was collected by filtration and re-dissolved in chloroform. The chloroform solution was then treated with a 1:1 (v/v) mixture of ethanol and H₂O to give a precipitate, which was collected by filtration and washed with

H₂O. The product was then dried under vacuum at 105°C and evaluated by NMR analysis to determine the migration ratio.

2.9. Synthesis of random substituted curdlan acetate propionate (ran-CDAcPr Ac:Pr=2:1)

Pre-dried curdlan (500 mg) was added to a 5:3:1 (v/v/v) mixture of TFAA, acetic acid and carboxylic acid (40 mL) under stirring at 50°C, and the resulting suspension was stirred at same temperature until it became clear. The mixture was then stirred for a further 30 min at same temperature before being poured into a saturated solution of sodium hydrogen carbonate (1 L). The resulting precipitate was collected by filtration and dissolved in chloroform. The chloroform solution was then treated with a 1:1 (v/v) mixture of ethanol and H₂O to give a precipitate, which was collected by filtration and washed with H₂O before being dried under vacuum condition at 105°C.

2.10. Nuclear magnetic resonance (NMR) spectroscopy

¹H NMR, ¹³C NMR, COSY, HSQC and HMBC analyses conducted on a JNM-A500 FT-NMR system (500 MHz, JEOL Ltd., Tokyo, Japan) at 25°C using CDCl₃ or pyridine-d₅ as a solvent. The degree of substitution (DS) was calculated based on the integration ratio of the acyl protons to the ring protons of the glucose unit.

$$DS_{\text{Acyl}} = 7I_{\text{Acyl-methetyl}}/3I_{\text{Ring proton}}$$

$$DS_{\text{Trityl}} = 7I_{\text{Trityl}}/15I_{\text{Ring proton}}$$

211 Where I is the integral of each peak.

212 **2.11. Gel permeation chromatography (GPC)**

213 The number and weight average molecular weight and polydispersity indices were
214 measured on a GPC system composed of a CBM-20A communications bus module,
215 DGC-20A3 degasser, LC-6AD liquid chromatograph, SIL-20A HT auto sampler, RLD-10A
216 refractive index detector and CTO-20A column oven (Shimadzu Corp., Kyoto, Japan).
217 Chloroform was used as the eluent at a flow rate 0.8 mL/min and the column oven
218 temperature was set to 40°C. Polystyrene standards were used to calibrate the molecular
219 weight.

220 **2.12. Thermogravimetric analysis (TGA)**

221 The thermal decomposition behaviors of the compounds prepared in the current study were
222 investigated using a Thermo plus TG8120 system (Rigaku Corp., Tokyo, Japan). Each sample
223 (5 mg) was put in platinum pan and heated from 30°C to 600°C at heating rate of 10°C/min
224 under an atmosphere of nitrogen gas.

225 **2.13. Differential scanning calorimetry (DSC)**

226 The glass transition, crystallization and melting behaviors of the different compounds
227 prepared in the current study were evaluated using a DSC 8500 system (PerkinElmer Co. Ltd,
228 Kanagawa, Japan). Samples were heated from 0°C to 300°C at a rate of 100°C/min, and then
229 cooled to 0°C at a cooling rate of 100°C /min. samples were the held at 0°C for 5 min before

being re-heated from 0°C to 300°C at a rate of 100°C /min. All of these measurements were carried out under an atmosphere of nitrogen gas.

3. Results and discussion

3.1. Tritylation of primary hydroxyl group and esterification of secondary hydroxyl group

Regioselectively substituted curdlan esters were synthesized to the method shown in Scheme 1. The tritylation of curdlan at the C6 primary hydroxyl groups of its glucose units was conducted under homogeneous conditions in a DMF/LiCl system. 6-*O*-Trityl-curdlan (CD6Tr) was founded to be insoluble in chloroform, pyridine and DMF solvents, which prevented its characterization by NMR spectroscopy.

The secondary hydroxyl groups at the C2 and C4 positions of the monomer units in CD6Tr were acetylated with acetic anhydride. The esterification of CD6Tr was carried out under heterogeneous conditions in pyridine with the substrate gradually dissolving to give a homogeneous solution as the reaction processed. The degree of substitution (DS) and substitution positions of the product were determined by NMR spectroscopy. ¹H NMR analysis revealed that the DS_{Ac} and DS_{Tr} values of this material were 2.0 and 1.0, respectively (Supplementary material. 1). Furthermore, the ¹³C NMR spectrum of this product contained two carbonyl peaks around 170 ppm, suggesting that only two of the hydroxyl groups had

249 been acetylated during this reaction (Figure 1). Unfortunately, no cross peaks were observed
 250 between the carbonyl carbons of the ester groups and the ring proton of the glucose unit by
 251 HMBC spectroscopy. Based on the overlap between the peaks belonging to H2 and H4 ring
 252 protons in the ^1H NMR spectrum and the regioselectivity of the tritylation process for the C6
 253 primary hydroxyl group, this product was assigned as 2,4-di-*O*-acetyl-6-*O*-trityl-curdlan
 254 (CD24Ac6Tr).

Scheme 1. Regioselective synthesis of substituted curdlan esters.

Figure 1. ^{13}C -NMR spectrum of CD24Ac6Tr.

3.2. Detritylation and unexpected acyl migration reaction of CD24Ac6Tr

CD24Ac6Tr was treated with HBr in chloroform to allow for the deprotection of the trityl protecting group from C6 hydroxyl group, which resulted in the formation of a yellow gel. ^1H -NMR analysis of this material revealed the presence of two methyl peaks belonging to the two acetyl groups (Supplementary material. 1). This result was confirmed by ^{13}C -NMR analysis, which showed two carbonyl peaks (Supplementary material. 2). Taken together, these results indicated that the acetyl groups only existed on two of the hydroxyl group of the detritylated CD24Ac6Tr material. It was difficult to calculate the DS_{Ac} of the detritylated CD24Ac6Tr material because the overlap of the peaks belonging to the hydroxyl protons and the ring protons. 2D-NMR HMBC spectroscopy was used to further examine the position of these two acetyl groups. As shown in Figure 2a, HMBC analysis revealed correlations between the two carbonyl carbons and ring protons at H2 and H6. These two carbonyl peaks were therefore assigned as C2 and C6 acetyl groups, respectively, and the molecular structure

271 of detritylated CD24Ac6Tr was determined to be 2,6-di-*O*-acetyl-curdlan (CD26Ac). A
272 comparison of the chemical shifts of the ring protons in CD24Ac6Tr with those found in
273 CD26Ac not only revealed considerable changes in the chemical shifts of the H6 and H6', but
274 also revealed a dramatic shift in the chemical shift of H4. This result indicated that the acetyl
275 group at the C4 position had migrated to the C6 position during the detritylation of
276 CD24Ac6Tr. Notably, there are no literature has been reported describing the occurrence of a
277 similar acyl migration process during the detritylation of other trityl-protected
278 polysaccharides, including cellulose (Iwata et al., 1992), amylose (Whistler & Hirase, 1961)
279 and chitin (Nishimura, Kohgo, Kurita & Kuzuhara, 1991). Given that curdlan consists of
280 β -(1 $\rightarrow$ 3)-D-glucose units, the distance between the C6 and C4 hydroxyl groups would be
281 much shorter than of those of the other polysaccharides listed above. This unique structural
282 characteristic of curdlan could therefore explain the unexpected acyl migration observed
283 during the detritylation of CD24Ac6Tr.

Figure 2. HMBC spectra of (a) CD26Ac and (b) CD26Ac4Pr.

3.3. Propionylation

The remaining free hydroxyl group of CD26Ac was reacted with propionyl chloride to give the corresponding propionic ester group. The DS_{Ac} and DS_{Pr} of this material were calculated to be 1.8 and 1.2, respectively (Table 1 and [Supplementary material. 1](#)). Although the DS_{Ac} of CD26Ac remains unknown, the ratio of the integrals for the C6 and C2 methyl peaks was determined to be 0.8:1 by 1H NMR analysis. The decrease in the DS_{Ac} value could be attributed to the partial hydrolysis of the C6 acetyl group (or C4 acetyl group) during the acid-mediated detritylation reaction. Three carbonyl peaks were observed in the ^{13}C NMR

spectrum of the product resulting from the propionylation of CD26Ac (**Supplementary material. 2**). Furthermore, HMBC analysis revealed two cross peaks between the carbonyl carbons of acetyl groups and the H2 and H6 ring protons, as well as one cross peak between the carbonyl carbon of propionyl group and the H4 ring proton (Figure 2b). These three carbonyl peaks were assigned as C2 acetyl, C6 acetyl and C4 propionyl groups, respectively. The molecular structure of product resulting from the propionylation of CD26Ac was therefore assigned as 2,6-di-*O*-acetyl-4-*O*-propionyl-curdlan (CD26Ac4Pr).

Table 1. Characterization of curdlan esters.

	DS			$M_w \times 10^{-4}$	PDI	$T_{d-50\%}$ (°C)	T_g (°C)	T_c (°C)	T_m (°C)
	DS _{Tr}	DS _{Ac}	DS _{Pr}						
CD	-	-	-	-	-	318	-	-	-
CDTAc	-	3.0	-	-	-	361	171	-	287(1 st run)
CD26Ac4Pr	0.0	1.8	1.2	3.3	1.74	358	128	199	230
ran-CDAcPr (Ac:Pr=2:1)	-	2.1	0.9	64.7	3.0	367	148	-	-
CDTPr	-	-	3.0	60	1.8	391	110	160	213

3.4. The effect of the acyl chain length of the ester on the acyl migration process

To confirm that the acyl migration occurred during the detritylation of the tritylated curdlan ester, we synthesized a series of CD24Acyl6Tr esters with different acyl chain lengths from propionate to decanoate and investigated their reactivity under detritylation conditions. The resulting products were acetylated to allow for their dissolution in chloroform for NMR analysis. The DS_{total} reached 3.0 in all of the products (**Supplementary material. 3**). ¹³C NMR spectroscopy of the material isolated following the acetylation of detritylated CD24Pr6Tr revealed five carbonyl peaks, which we were assigned to the C4 and C6 acetyl groups, and

the C2, C4 and C6 propionyl groups by HMBC spectroscopy (Figure 3). The propionyl group was found on all of the hydroxyl groups in this case, suggesting that only a small proportion of the acetyl group at the C4 position had migrated to the C6 position during the detritylation of CD24Pr6Tr. Similar results were also observed following the detritylation of the other CD24Acyl6Tr substrate with acyl groups from butyrate to decanoate. The DS_{acyl} distributions of these compounds are listed in Table 2. In the detritylated product of CD24Ac6Tr, almost all C4 acetyl groups migrated to the C6 position. In the detritylated product of CD24Pr6Tr, the DS_{Pr} of the C4 position was 0.4, suggesting that 60% of C4 propionyl groups had migrated to the C6 position. For the detritylated products of CD24Bu6Tr to CD24De6Tr, the DS_{Acyl} values of the C4 positions were 0.5, suggesting that only 50% of acyl groups in this group (i.e., chain lengths from butyrate to decanoate) had migrated during the detritylation reaction.

Table 2. DS dispersion of detritylated product of CD24Acyl6Tr.

starting material	C2	C4	C6
CD24Ac6Tr	0.9	0.0	0.9
CD24Pr6Tr	1.0	0.4	0.6
CD24Bu6Tr	1.0	0.5	0.5
CD24Va6Tr	1.0	0.5	0.5
CD24He6Tr	1.0	0.5	0.5
CD24Oc6Tr	1.0	0.5	0.5
CD24De6Tr	1.0	0.5	0.5

Figure 3. HMBC spectra of further-acetylated detritylated CD24Pr6Tr.

3.5. Mechanism of this unexpected acyl migration reaction

The acyl migration processes similar to the acyl migration observed in this study involves the movement of an acyl group from a secondary hydroxyl group to a relatively stable primary hydroxyl group, have also been reported in glycerol derivatives (Van Lohuizen & Verkade, 1960; Jackson & Lundberg, 1963; Krabishch & Borgstrom, 1965; Kodali, Tercyak, Fahey & Small, 1990) and glucosides (Iddon et al., 2011). Acyl migration occurs in glycerol esters at high temperatures or under acidic or basic conditions, as well as during the detritylation of trityl glycerol esters. The mechanism of acyl migration in glycerol esters involves the initial attack of a lone pair of electrons on the oxygen atom of the primary hydroxyl group to the carbonyl carbon of the acyl group positioned on the secondary hydroxyl, leading to the formation of a five- or six-membered ring intermediate. The ring is

337 then opened by the cleavage of the single bond between the carbonyl carbon and the oxygen
338 of the secondary hydroxyl group. The acyl group then migrates to the primary hydroxyl
339 position (Krabishch & Borgstrom, 1965). Acyl migration occurs via a similar mechanism in
340 glucoside. If the acyl groups in the curdlan esters migrated in the same way as these
341 compounds described above, the migration mechanism would start with the attack of the lone
342 pair of electrons on the C6 hydroxyl group of CD24AcylO on the carbonyl carbon of the acyl
343 group. This would lead to the formation of a six-membered ring between the C4 and C6
344 positions following the cleavage of trityl group at C6 position. There are two possible
345 positions for this cleavage of the six-membered ring intermediate, including (i) the single
346 bond between the carbonyl carbon and the C4 hydroxyl group, which would result in the
347 migration of the acyl group to the C6 hydroxyl; and (ii) the single bond between the carbonyl
348 carbon and the C6 hydroxyl group, which would result in the acyl group remaining at C4
349 position (Scheme 2a). According to this mechanism, the rate of migration would be
350 determined by the nucleophilicities of the hydroxyl oxygens at the C4 and C6 positions,
351 which would be unaffected by the acyl chain length. The migration ratio would therefore be
352 expected to remain constant regardless of the length of the acyl group. The CD24Acyl6Tr
353 substrates with acyl chain lengths from butyrate to decanoate showed a constant migration
354 ratio of 50%, indicating that these substrates were following the mechanism described above.
355 However, the migration rates in CD24Ac6Tr and CD24Pr6Tr were approximately 100% and

356 60%, respectively, indicating that the migration occurred via a different mechanism in the
357 substrates.

358 The acyl migration reaction of CD24Ac6Tr and CD24Pr6Tr could potentially occur via
359 another mechanism that is especially sensitive to steric hindrance. Scheme 2b shows an
360 alternative mechanism for the acyl migration reaction in CD24Ac6Tr and CD24Pr6Tr.
361 According to this mechanism, the lone pair on the oxygen atom of the C6 trityl ether would
362 attack the carbonyl carbon of the acyl group at C4, to give a six-membered ring intermediate.
363 The trityl group would then leave with the concomitant cleavage of the single bond between
364 the carbonyl carbon and the oxygen atom at C4-oxygen, completing the migration of the acyl
365 group to C6 position. The formation of the six-membered ring intermediate in this
366 mechanism would be affected by the hindrance between the trityl group and the acyl chain,
367 making it difficult for increasing bulky acyl group undergo this migration. The acetyl group
368 would experience very little steric hindrance because of its small size, resulting in the
369 observed 100% migration. The migration rate would be decrease for the propionic group
370 because of the increase in steric hindrance caused by the increasing size of the acyl chain
371 length.

(a) In the case of R= Bu to De.

(b) In the case of R= Ac and Pr

Scheme 2. Acyl migration mechanism of (a) in the case of Bu to De, (b) in the case of Ac to Pr.

3.6. Thermal properties

The thermal degradation behavior of CD26Ac4Pr was analyzed by TGA. The temperature at which the remaining weight reached 50% ($T_{d-50\%}$) and the degradation curves are shown in Table 1 and Figure 4a, respectively. CD26Ac4Pr reached $T_{d-50\%}$ at 358°C. The thermal decomposition curve of CD26Ac4Pr shifted to a higher temperature compared with neat curdlan. It is well known that levoglucosan is formed as the major volatile product of curdlan degradation (Richards & Shafizadeh, 1982) and that the esterification of this curdlan efficiently hinders the formation of levoglucosan and improves the thermal stability of this material (Marubayashi et al., 2014). The regioselective esterification of curdlan also results in an increase in its $T_{d-50\%}$ value. The $T_{d-50\%}$ value of CD26Ac4Pr and a randomly substituted

curdlan acetate propionate (ran-CDAcPr) were found to be identical, suggesting that the thermal decomposition behaviors of curdlan esters are independent of the ester group distribution in curdlan acetate propionate.

The thermal transition behavior of CD26Ac4Pr was analyzed by DSC. The esterification of curdlan can have a considerable impact on its melting behavior. The melting temperature of the curdlan esters decreased with increasing acyl chain length. For example, curdlan acetate and curdlan propionate showed a high melting temperature of greater than 200°C (Marubayashi et al., 2014). In the second run thermogram of DSC analysis of CD26Ac4Pr, the melting endothermic peak (T_m) and crystallization exothermic peak (T_c) were observed at 230°C and 199°C, respectively. CD26Ac4Pr showed T_c and T_m transition, whereas ran-CDAcPr showed no such transitions (Figure 4b), suggesting that the crystallization of the curdlan hetero-esters was accelerated by their regioselective esterification. It has been reported that the crystallization of the regioselectively substituted cellulose ester 2,3-di-*O*-acetyl-6-*O*-propionyl-cellulose can be controlled by varying the nature of the ester groups at the C2 and C3 positions (Iwata et al., 1994). The different thermal behaviors observed for the different regioisomer of the substituted curdlan esters (CD26Ac4Pr) and ran-CDAcPr indicated that the formation of crystals in the curdlan hetero-ester had been improved by the regular substitution of the ester group, which could be attributed to the crystallization-promoting nature of specific ester groups in the curdlan hetero-esters. The T_g ,

T_c and T_m transitions of CD26Ac4Pr were observed between those of curdlan triacetate (CDTAc) and curdlan tripropionate (CDTPr). CD26Ac4Pr, with a T_m value over 200°C, existed as a high melting temperature material. To clarify the relationship between the crystallinity and substitution position of the ester group, we would need to conduct X-ray diffraction measurements to analyze the crystal structure of these regioselectively substituted curdlan hetero esters.

Figure 4. (a) TGA and (b) DSC thermograms of CD and CD hetero-esters.

4. Conclusion

We regioselectively synthesized a series of substituted curdlan esters using a 6-*O*-tritylation strategy to differentiate the primary and secondary hydroxyl groups and observed a unique acyl migration in detritylation of tritylated curdlan esters which was first found in curdlan among polysaccharides. The primary hydroxyl group at the C6 position of curdlan was regioselectively protected with a trityl group. The subsequent reaction of this trityl-protected

material with acetic anhydride resulting in the regioselective acetylation of the C2 and C4 hydroxyl groups to give 2,4-di-*O*-acetyl-6-*O*-trityl curdlan. During the subsequent detritylation of this material in chloroform with HBr, we observed the detritylation of the trityl group together with the migration of the acetyl group from the C4 position to the C6 position to give 2,6-di-*O*-acetyl-curdlan. This acyl migration was found to be influenced by the acyl chain length, with an acetyl group at the C4 position giving 100% migration. In contrast, the addition of larger propionyl group at same position resulted in only 60% migration. Furthermore, this downward trend continued, with only 50% of the acyl groups from butyryl to decanoyl undergoing the migration. We have proposed two possible mechanisms for this acyl migration process in curdlan esters. The free C4 hydroxyl group of 2,6-di-*O*-acetyl-curdlan was propionylated to give 2,6-di-*O*-acetyl-4-*O*-propionyl-curdlan (CD26Ac4Pr). A comparison of the thermal behavior of CD26Ac4Pr with that of ran-CDAcPr revealed that the substitution position of the curdlan ester has no influence on its thermal decomposition. However, the regioselectively substituted curdlan ester existed as a crystalline polymer despite the fact that the randomly-substituted curdlan ester was amorphous, suggesting that this regioselective substitution enhanced the crystallization of this material.

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Caption of figure

Scheme 1. Regioselective synthesis of substituted curdlan ester.

Scheme 2. Acyl migration mechanism of (a) in the case of Bu to De, (b) in the case of Ac to Pr.

Table 1. Characterization of curdlan eaters.

Table 2. DS distribution of detritylated product of CD24Acyl6Tr.

Figure 1. ¹³C-NMR spectrum of CD24Ac6Tr.

Figure 2. HMBC spectra of (a) CD26Ac and (b) CD26Ac4Pr.

Figure 3. HMBC spectrum of further-acetylated detritylated CD24Pr6Tr.

Figure 4. (a) TGA and (b) DSC thermograms of CD and CD hetero-esters.

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Table of contents graphic

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Unexpected acyl migration in curdlan esters