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Glycan ester deacylation by TBAOH or TBAF: Regioselectivity vs. polysaccharide structure

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ABSTRACT

Tetrabutylammonium fluoride (TBAF) and tetrabutylammonium hydroxide (TBAOH) have been found to mediate regioselective deacylation of cellulose esters, with unexpected selectivity for removal of acyl groups at the more hindered secondary O-2/3 positions. This simple, efficient, one-step process triggers our investigation of TBAF/TBAOH deacylation on other glycan (amylose, curdlan, dextran, pullulan and glucomannan) esters to examine the generality of this reaction and the impact of glucan and glucomannan structure on deacylation regioselectivity. Remarkably regioselective O-2/3 deacylation was observed with amylose triesters, while moderately regioselective O-2/4 deacylation of curdlan triester occurred. No regioselectivity was observed with dextran triester, as predicted due to the lack of primary OH groups. We observed deacylation of pullulan and glucomannan triesters, but due to the complexity of the partially substituted products have not yet been able to determine the deacylation regioselectivity for these glycan esters.

1. Introduction

Polysaccharides are ubiquitous biopolymers with a wide variety of structures, properties and functions. Esterification of polysaccharides has been a reliable and useful modification strategy, providing ready access to a broad variety of bio-based materials with desirable properties (Heinze, Liebert, & Koschella, 2006). Though polysaccharide esterification has been known for over a century, it remains very challenging to control the position of acylation of polysaccharides. Regioselective substitution of polysaccharides is difficult to perform due to the slight reactivity differences among different positions and the generally poor organic solubility of polysaccharides. The most powerful approach for regioselective polysaccharide derivative synthesis has thus far been the protection/deprotection approach (Fox, Li, Xu, & Edgar, 2011). However, this method is practically limited to preparation of lab scale samples due to the multiple steps involved, low overall yields, expensive reagents, and the potential for inadequate reactivity of some of the protected intermediates. Those issues motivate us to seek out an efficient, broadly applicable procedure for synthesis of regioselectively substituted polysaccharide derivatives.

In the 1990s researchers investigated the deacylation of fully substituted cellulose esters by aliphatic amines in order to synthesize regioselectively substituted derivatives but the best regioselectivity (O-6 vs. O-2/3) obtained was only around ~60% (Philipp et al., 1995; Wagenknecht, 1996). Recently Xu and Edgar (2012) have discovered that tetrabutylammonium fluoride (TBAF) smoothly deacylates cellulose esters, with unexpected regioselectivity, and a remarkable predominance of deacylation at the more hindered secondary positions O-2 and O-3. This simple one-step process produces cellulose-6-*O*-esters with high regioselectivity, which can also be easily modified into cellulose-2,3-*O*-A-6-*O*-B-triesters. The mechanism of this unexpected reaction has been investigated by methods including kinetic isotope effect (KIE) studies (Zheng, Gandour, & Edgar, 2013a). Deacylation at the secondary O-2/3 positions occurs by an E1cB mechanism involving a ketene intermediate, while deacylation at the primary O-6 occurs by a different mechanism, general base catalysis involving a tetrahedral intermediate. A further investigation of reaction scope explored the influences of factors including solvent, temperature and water content upon deacylation regioselectivity and provided results consistent with the mechanistic proposals above (Zheng, Gandour, &

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Edgar, 2013b). Reactions of cellulose esters (acetate, propionate, butyrate, hexanoate and benzoate) with TBAF in DMSO at 50 °C for 18 h provided the most efficient conditions for regioselective deacylation at O-2/3. Reaction with the strongly basic tetrabutylammonium hydroxide (TBAOH) has been shown to afford similarly regioselective deacylation of cellulose esters (Zheng, Gandour, & Edgar, 2014), but with the advantages vs. TBAF of milder reaction conditions, less expensive reagent, and the potential for easier recycling by simple ion exchange. We have hypothesized that chelation between the tetrabutylammonium cation and the ester oxygen atoms of the vicinal 2,3-acyl groups is responsible for the observed regioselectivity of TBA-catalyzed deacylation.

We felt that investigating the possible extension of this chemistry to esters of other glucans and glucomannans might be of interest, not only because of the potential value of a general method for regioselective synthesis of derivatives of these polysaccharides. By studying deacylation of these other polysaccharide esters, we also hoped to gain further insight into the source of the unusual regioselectivity of these deacylation reactions. For example, deacylation of esters of amylose (a component of plant starch; Fuentes-Zaragoza, Riquelme-Navarrete, Sánchez-Zapata, & Pérez-Álvarez, 2010) would give information about the influence of anomeric stereochemistry upon deacylation selectivity, since amylose differs from cellulose nearly exclusively in that it has α-1 → 4 rather than β-1 → 4 linkages. On the other hand, deacylation of esters of unbranched dextran would provide an example in which there were no primary alcohol esters present, due to the α-1 → 6 backbone linkages of dextran; dextran is a bacterial polysaccharide that is often branched in nature, but in some cases, including the material used here, is unbranched. Dextran is highly water-soluble and finds wide utility in injectable formulations for human use (Åberg, Hedner, & Bergentz, 1979; Maia et al., 2009). What would be the regioselectivity of deacylation in the absence of primary alcohol esters? Esters of pullulan, a bacterial polysaccharide useful in buccal delivery formulations and food applications (Hosseinkhani, Aoyama, Ogawa, & Tabata, 2002; Leathers, 2003), would provide an internal comparison of results from deacylation of monosaccharides containing α-1 → 4 vs. α-1 → 6 linkages, since pullulan has a maltotriose (glucose trimer with internal α-1 → 4 linkages) repeat unit connected by α-1 → 6 linkages.

The absence of a vicinal diol structure in curdlan, due to its β-1 → 3 linkages, would test our previously expressed hypothesis that complexation of the tetralkylammonium ion with the 2,3-vicinal diester of cellulose esters is a primary driving force for the observed selectivity of that reaction for deacylation of the 2,3-esters. Curdlan is a glucan which is also a bacterial polysaccharide, produced by the bacterium *Alcaligenes faecalis* var. myxogenes, and is water-insoluble (Zhang & Edgar, 2014a). Curdlan is used in food applications based on its excellent thermal-dependent gelation properties, ability to mimic fat/meat mouth-feel, and ability to modify rheological properties of sauces and dairy products (McIntosh, Stone, & Stanisich, 2005). Finally, glucomannan esters would provide interesting information as well, since the 2,3-diol in mannose has the *cis* orientation, vs. the *trans* orientation in cellulose, amylose, dextran and pullulan. Glucomannan consists of β-1,4-linked D-glucose and D-mannose; in the sample we employed, the glucose:mannose ratio was 1.6:1 (Katsuraya et al., 2003). Water-soluble glucomannan is frequently obtained from plant seeds, finds utility as a natural gum in foods, and is also used in films, coatings, and drug delivery systems (Yu, Huang, & Xiao, 2006). We anticipated that deacylation of glucomannan esters would provide understanding of the potential influence of 2,3-diester orientation upon the proposed cation coordination mechanism.

We report herein our results with regard to extent and regioselectivity of deacylation of each of these triesters with TBAF or TBAOH, and the implications of those results for the generality and mechanism of these methods for synthesis of regioselectively substituted glucan and glucomannan esters.

2. Experimental

2.1. Materials

Amylose from potato (DP ~ 13,000, Sigma Co.), unbranched dextran (DP ~ 56–68, Sigma Co.) and curdlan (DP ~ 6173, Wako Chemicals) were purchased and dried under vacuum at 40 °C overnight prior to use. Pullulan (DP ~ 1235) was purchased from Hayashibara Company (Okayama, Japan) and was dried under vacuum at 120 °C overnight prior to use. Konjac glucomannan (KGM, DP ~ 1475) (Propol® A) was kindly provided by Shimizu Chemical Company (Hiroshima, Japan). Tetrabutylammonium fluoride (TBAF, approximately as trihydrate), tetrabutylammonium hydroxide (TBAOH 40 wt% in water), 4-dimethylaminopyridine (DMAP), lithium chloride (LiCl), pyridine (anhydrous, 99%, AcroSeal®), tetrahydrofuran (THF, 99.8%, extra dry, AcroSeal®), acetic anhydride (Ac_2O, 99+%) and propionic anhydride (Pr_2O, 97%) were purchased from Acros Organics and used as received. *N,N*-dimethylacetamide (DMAc, reagent grade, Fisher) and dimethyl sulfoxide (DMSO, HPLC grade, Fisher) were stored over 4 Å molecular sieves. Iodine (I_2, Fisher), methanol (HPLC grade, Fisher), ethanol (HPLC grade, Fisher) and sodium thiosulfate (anhydrous, J.B. Baker Inc.) were used as received.

2.2. Measurements

^1H, ^{13}C, HMBC, and COSY NMR spectra were obtained on a Bruker Avance II 500 MHz spectrometer in $CDCl_3$ or DMSO-d_6 at room temperature or 50 °C, with number of scans of 32, 15,000, 19,200 and 9400, respectively. Total and partial DS (degree of substitution) values of glycan ester products after peracylation were determined by calculating the ratios of acetyl or propionyl proton integrals to those of the backbone hydrogens (Liebert, Hussain, & Heinze, 2005; Xu & Edgar, 2012).

2.3. Preparation of glycan triesters

2.3.1. Preparation of amylose and curdlan triesters

Dissolution of amylose or curdlan in DMAc/LiCl was adapted from a method previously reported for cellulose dissolution (Edgar, Arnold, Blount, Lawniczak, & Lowman, 1995; Zhang & Edgar, 2014b). A mixture of dried amylose or curdlan (1 g, 6.2 mmol anhydroglucose unit (AGU)) and DMAc (37.5 mL) was kept at 150 °C for 26 min with vigorous stirring under nitrogen. Anhydrous LiCl (1.88 g) was added, and the mixture was stirred at 165 °C for 8 min. DMAc (10 mL) was distilled off to facilitate water removal. The slurry was cooled to room temperature while being stirred overnight, during which time dissolution occurred.

Synthesis of amylose or curdlan triesters was adapted from a previously published procedure (Xu & Edgar, 2012; Zheng et al., 2013a, 2013b). Amylose tripropionate (ATP) or curdlan triacetate (CTA) was prepared by adding DMAP (20 mg), pyridine (5.8 mL, 10 eq/AGU) and propionic anhydride (7.9 mL, 10 eq/AGU) or acetic anhydride (5.8 mL, 10 eq/AGU) to the amylose or curdlan solution. The solution was kept at 80 °C for 24 h and then added slowly to 500 mL deionized water under vigorous stirring. The crude product was isolated by filtration, and then re-dissolved in 10 mL chloroform. This solution was added slowly with rapid stirring to 50 mL of ethanol. After filtration and washing with ethanol and water several times, the sample was dried under vacuum at 40 °C overnight. DS_{Pr}(ATP) = 3.0 and DS_{Ac}(CTA) = 3.0 by ^1H NMR ($CDCl_3$): ATP: δ 0.86–1.26 (CH_3–propionate), δ 2.02–2.54 (CH_2–propionate),

δ 3.70–5.52 (amylose backbone); CTA: δ 1.84–2.19 (CH_3–acetate), δ 3.49–5.07 (curdlan backbone). Yields: ATP 80%, CTA 89%.

2.3.2. Preparation of dextran triester

Synthesis of dextran triacetate (DTA) was performed by a previously published procedure (Hussain et al., 2010). Iodine (0.5 g) was added to a three-necked round bottom flask. Acetic anhydride (5.8 mL, 10 eq/AGU) was added and the reaction mixture was kept under stirring for 15 min. Pre-dried dextran (1.0 g, 6.2 mmol) was added and the resulting mixture was refluxed at 50 °C for 3 h under nitrogen. The excess of iodine (catalyst) was removed by adding a saturated aqueous sodium thiosulfate solution to the reaction mixture. The thus formed precipitate was filtered off, washed twice with methanol and cold water, and then dried under vacuum at 40 °C overnight. DS_{Ac}(DTA) = 3.0 by ^{1}H NMR ($CDCl_3$): δ 1.85–2.18 (CH_3-acetate), δ 3.39–5.65 (dextran backbone). Yield: 92%.

2.3.3. Preparation of pullulan triester

Synthesis of pullulan triacetate (PTA) was performed by a previously published procedure (Teramoto & Shibata, 2006). Pullulan (2 g, hydroxyl group 37 mmol) was dissolved in DMAc (50 mL). Pyridine (8.8 mL, 10 eq/mol anhydroglucose unit (AGU) of pullulan,) was added to the solution, followed by addition of acetyl chloride (10 mL, 10 eq/mol AGU of pullulan). The mixture was stirred at 60 °C for 20 h, and then the solution was added slowly to ethanol (500 mL) under vigorous stirring. The formed precipitate was filtered and washed with ethanol several times, and dried under vacuum at 40 °C overnight. DS_{Ac}(PTA) = 3.0 by ^{1}H NMR (DMSO-d_6): δ 1.80–2.20 (CH_3-acetate), δ 3.42–5.67 (pullulan backbone). Yield: 82%.

2.3.4. Preparation of glucomannan triester

Synthesis of glucomannan triacetate (GTA) was performed as previously described (Enomoto-Rogers, Ohmomo, & Iwata, 2013). Briefly, glucomannan (0.5 g) was pretreated by dissolving in water (50 mL) and freeze–drying. A pre-mixed solution of acetic acid (20 mL) and trifluoroacetic anhydride (TFAA, 20 mL), which had been stirred at 50 °C for 20 min, was immediately added to the freeze-dried glucomannan. The reaction solution was stirred at 50 °C for 1 h under nitrogen. After cooling to room temperature, the solution was slowly added to ethanol (500 mL). The crude product was collected by filtration, re-dissolved in chloroform (10 mL), and re-precipitated into ethanol for further purification. The sample was dried under vacuum at 40 °C overnight. DS_{Ac}(GTA) = 3.0 by ^{1}H NMR ($CDCl_3$): δ 1.86–2.10 (CH_3-acetate), δ 3.36–5.32 (glucomannan backbone). Yield: 84%.

2.4. General procedures for deacylation of glycan triesters

2.4.1. Tetrabutylammonium fluoride deacylation of glycan triesters

To a solution of glycan triester in DMSO or THF (40 mL/g of esters) was added TBAF trihydrate (4 eq/mol glucopyranosyl group), and the reaction solution was kept at 50 °C for 24 h. For CTA, PTA and GTA, the solution was added slowly to water (250 mL). For ATP, the solution was transferred to 3500 g/mol MWCO dialysis tubing that was then placed in a large beaker containing water for 3 days. For DTA, the solution was added slowly to methanol (250 mL). The thus formed precipitate was collected by filtration, washed several times with water or methanol, and dried under vacuum at 40 °C overnight. The samples were further perpropionylated or peracetylated (details below) for DS determination.

2.4.2. Tetrabutylammonium hydroxide deacylation of glycan triesters

To a solution of glycan triester in DMSO or THF (40 mL/g of ester) was added TBAOH (2 eq/mol glucopyranosyl group), and the reaction solution was kept at room temperature for 1 h or at 50 °C for 24 h. For CTA, PTA and GTA, the solution was added slowly to water (250 mL). For ATP, the solution was transferred to 3500 g/mol MWCO dialysis tubing that was then placed in a large beaker containing water for 3 days. For DTA, the solution was added slowly to methanol (250 mL). The thus formed precipitate was collected by filtration, washed several times with water or methanol, and dried under vacuum at 40 °C overnight. The samples were further perpropionylated or peracetylated (details below) for DS determination.

2.5. General procedure for peracylation of deacylated glycan esters

Deacylated products were peracetylated or perpropionylated for easier NMR analysis, according to previously published procedures (Liebert et al., 2005; Xu & Edgar, 2012). DMAP (15 mg) and acetic anhydride (4 mL) or propionic anhydride (4 mL) were added to the solution of deacylated glycan esters (0.3 g) in pyridine (4 mL). After stirring at 80 °C for 24 h, the crude product was obtained by precipitation into water or ethanol (200 mL) and washed several times by water or ethanol. Re-dissolving the crude product in chloroform and re-precipitating it into ethanol further purified the product. The sample was dried under vacuum at 40 °C overnight for analysis. The total and partial DS values for the peracetylated or perpropionylated products were determined according to Eqs. (1) and (2) by ^{1}H NMR.

$$DS_{\text{ester}} = 3 - \frac{7I_{H,\ \text{acetyl or propionyl}}}{3I_{H,\ \text{AGU}}};\quad I = \text{integral} \tag{1}$$

$$DS_{\text{ester}(n)} = 1 - \frac{7I_{H,\ \text{acetyl or propionyl}\ (n)}}{3I_{H,\text{AGU}}};$$

$$I = \text{integral},\quad n = \text{Position}\ \ 2,\ \ 3\ \ ,4\ \ \text{or}\ \ 6 \tag{2}$$

3. Results and discussion

3.1. Deacylation of amylose tripropionate (ATP)

Due to the poor solubility of amylose triacetate, ATP was chosen as the deacylation substrate. Conditions chosen were those that had worked well with cellulose esters (4 eq TBAF, THF solvent, 50 °C, 24 h) (Xu & Edgar, 2012). The product amylose propionate had DS_{Pr} of approximately 1.03, as calculated by ^{1}H NMR peak integration (Fig. 1). Two strong resonances for the acetyl methyl groups appeared at δ 1.9–2.1. To further confirm the positions of ATP deacylation and calculate the partial DS of each position, we carried out a heteronuclear multibond correlation NMR experiment (HMBC), in which the cross peaks between ester carbonyls and the nearest ring hydrogens of the AGU (three-bond correlation) are diagnostic of the position of substitution. Two clear correlation peaks were observed between the two diastereotopic H-6 resonances at 4.45 and 4.19 ppm and the propionate carbonyl ^{13}C resonance at 174 ppm (Fig. 2). The calculated partial $DS_{Ac(2)}$ = 0.97 and $DS_{Ac(3)}$ = 0.98 supported nearly perfectly regioselective deacylation of ATP at O-2/3 (98% regioselectivity, Table 1, entry 1).

Reactions of ATP with TBAOH were performed to study the impact on regioselectivity of varying reaction time, temperature and reagent molar ratios (Table 1). Treatments of ATP with 1 eq/AGU TBAOH (entry 2–4) provided incomplete deacylation of propionyl groups at O-2/3. Treatments of ATP with 2 eq/AGU TBAOH (entry 5–7) gave highly regioselective deacylation, affording a product with residual propionate DS_{Pr} 0.92–0.99. Under the best conditions TBAOH deacylation was also nearly perfectly

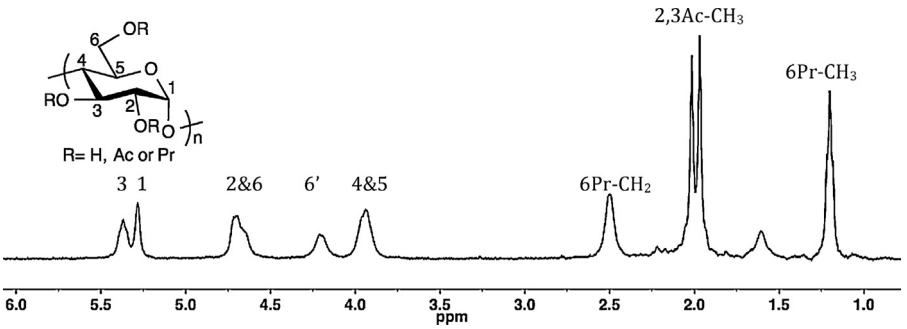

Fig. 1. ^{1}H NMR spectrum of the product of amylose tripropionate deacylation by TBAF (24 h, THF, 50 °C), after peracetylation.

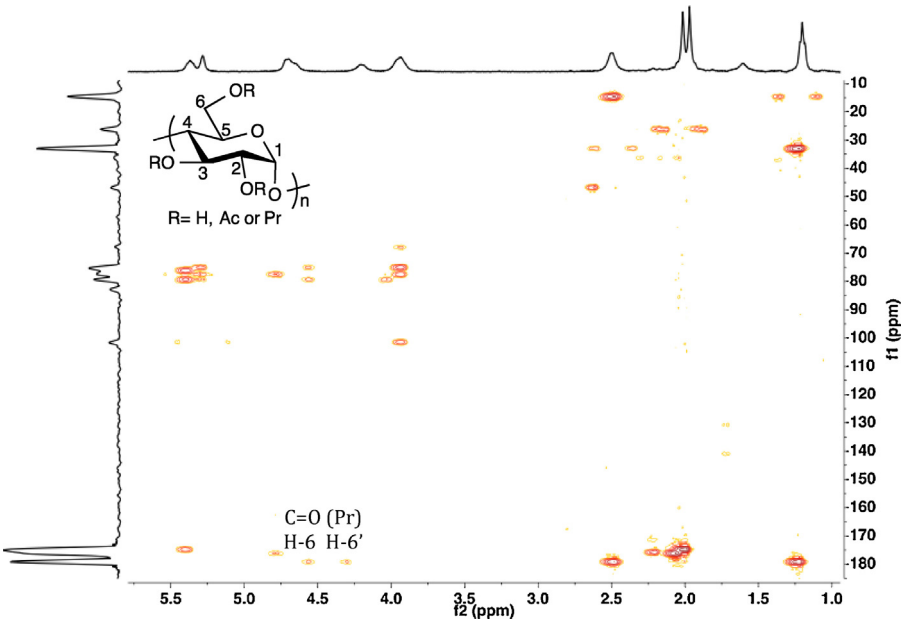

Fig. 2. HMBC NMR spectrum of the product of amylose tripropionate deacylation by TBAF (24 h, THF, 50 °C), after peracetylation.

regioselective (99% regioselectivity). The ^{1}H NMR spectra showed the absence of propionate esters at O-2/3, similar to the results with TBAF.

3.2. Deacylation of curdlan triacetate (CTA)

Curdlan triacetate (DS_{Ac} 3.0) was subjected to deacylation with TBAF in THF (4 eq, 50 °C, 24 h). The precedent of preference for TBAF deacylation of cellulose and amylose esters at the secondary positions might lead one to expect selective TBAF deacylation of curdlan triacetate at the O-2/4 positions; however, the absence of the vicinal diester functionality in curdlan triacetate (vs. the presence of the vicinal 2,3-diacetate groups in cellulose and amylose triacetates) might be expected to reduce regioselectivity if cation coordination by the vicinal diester were, as we have hypothesized, the driving force of regioselectivity. The product of TBAF deacylation of curdlan triacetate, upon perpropionylation, displayed peaks around δ 2.1–2.6 and δ 0.9–1.3 in the proton NMR spectrum (Fig. 3). We assigned these resonances to propionyl methylene and methyl groups, indirectly indicating the expected acetate deacylation with DS_{Ac} of 1.79. HMBC was performed to assign the positions of substitution and thus reveal deacylation regioselectivity. The HMBC spectrum showed a clear correlation peak between the H-2/4 resonances at 4.7 and the propionate carbonyl ^{13}C resonance at 172 ppm

Table 1
Results of TBAF/TBAOH deacylation of ATP in THF.

Entry	TBAF/TBAOH (eq/AGU)[a]	Temp. (°C)	Time (h)	$DS_{Pr(6)}$	$DS_{Pr(2+3)}$	$DS_{Pr(total)}$	PR[b] (%)
0	0	–	–	1.00	2.00	3.00	–
1	4	50	24	0.98	0.05	1.03	98
2	1	50	24	0.98	0.72	1.70	97
3	1	50	1	0.98	0.88	1.86	96
4	1	RT	1	0.99	1.03	2.02	98
5	2	50	24	0.99	0.03	1.02	99
6	2	50	1	0.95	0.02	0.97	95
7	2	RT	1	0.92	0.07	0.99	92

[a] Entry 1: TBAF Entry 2–7: TBAOH.

[b] Percent regioselectivity (PR) $= \frac{DS_{2+3(S)}-DS_{2+3(P)}}{DS_{total(S)}-DS_{total(P)}} - \frac{DS_{6(S)}-DS_{6(P)}}{DS_{total(S)}-DS_{total(P)}}$; S = starting amylose tripropionate, P = product.

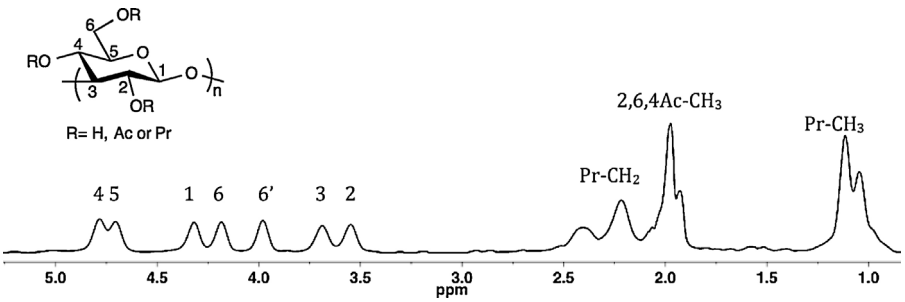

Fig. 3. ^{1}H NMR spectrum of the product of curdlan triacetate deacylation by TBAF (24 h, THF, 50 °C), after perpropionylation.

(Fig. 4). Due to the overlapping of acetyl protons at different positions, it was difficult to calculate the partial DS values of acetyl residues at O-2/4/6 after deacylation. Interestingly, the ^{1}H NMR spectrum of the unperpropionylated product after TBAF treatment (Fig. S3) demonstrated a well-separated acetyl peak for each position even though this particular sample contained a small amount of tetrabutylammonium salt contaminant. Partial DS values were calculated as $DS_{Ac(2)}$ = 0.50, $DS_{Ac(4)}$ = 0.51 and $DS_{Ac(6)}$ = 0.78 with a percent regioselectivity (PR) around 64%.

Reaction of CTA with 2 eq. TBAOH at room temperature for 1 h provided similar deacylation extent and regioselectivity to that afforded at longer time and higher temperature (50 °C for 24 h), indicating that deacylation is rapid even under mild conditions. Three separate peaks around δ 1.97–2.13 (Fig. 5) arise from the residual acetyl methyl groups at O-2/4/6 with $DS_{total(Ac)}$ of 1.19. Partial DS_{Ac} values for each position were determined by the ratio of the partial acetyl resonances (O-2 δ 2.09–2.13, O-6 δ 2.03–2.08, O-4 δ 1.97–2.01) to the integrals of the curdlan backbone protons. The resultant curdlan acetate with $DS_{Ac(6)}$ = 0.44, $DS_{Ac(2)}$ = 0.28, and $DS_{Ac(4)}$ = 0.30 indicated slight preference for deacylation at O-2/4 over O-6, with almost equal levels of deacylation occurring at the two secondary positions (O-2 and O-4). This is consistent with the hypothesis that coordination of the cation by vicinal ester groups is necessary for maximum selectivity for secondary alcohol ester deacylation.

3.3. Deacylation of dextran triacetate (DTA)

TBAF deacylation of the triacetate ester of unbranched dextran (DTA, DS_{Ac} 3.0) was carried out under the same conditions as for amylose tripropionate and curdlan triacetate (4 eq TBAF, THF solvent, 50 °C for 24 h). The resulting dextran acetate had DS_{Ac} 2.05 (Fig. 6). HMBC analysis combined with ^{1}H NMR integration again provided information about regioselectivity. Due to the overlapping resonances of the O-2/4 acetyl groups, we were not able to ascertain partial DS values for every individual position, but were able to determine that $DS_{Ac(2+4)}$ = 1.28 and $DS_{Ac(3)}$ = 0.61. This is consistent with the interpretation that deacylation of the acetyl groups at O-2 and O-4 proceeded to the same extent as that observed at O-3, since $DS_{Ac(2+4)}$ is almost twice $DS_{Ac(3)}$. These results support our hypothesis that reaction rates at all three ester groups of unbranched dextran triacetate should be similar, since all three are esters of secondary alcohols, and since vicinal diester relationships exist between both the O-2 and O-3 positions, and the O-3 and O-4 positions.

We carried out deacylation treatments in which dextran triacetate was exposed to TBAOH, examining the effect of TBAOH/DTA stoichiometry (Table 2). After 1 h reaction of DTA with 1 eq TBAOH in THF (entry 2), the DS acetate was reduced from 3.0 to 1.93 with $DS_{Ac(2+4)}$ = 1.28 and $DS_{Ac(3)}$ = 0.65, similar results as for TBAF deacylation. Using 2 eq TBAOH, the residual DS_{Ac} at O-2/4 and O-3

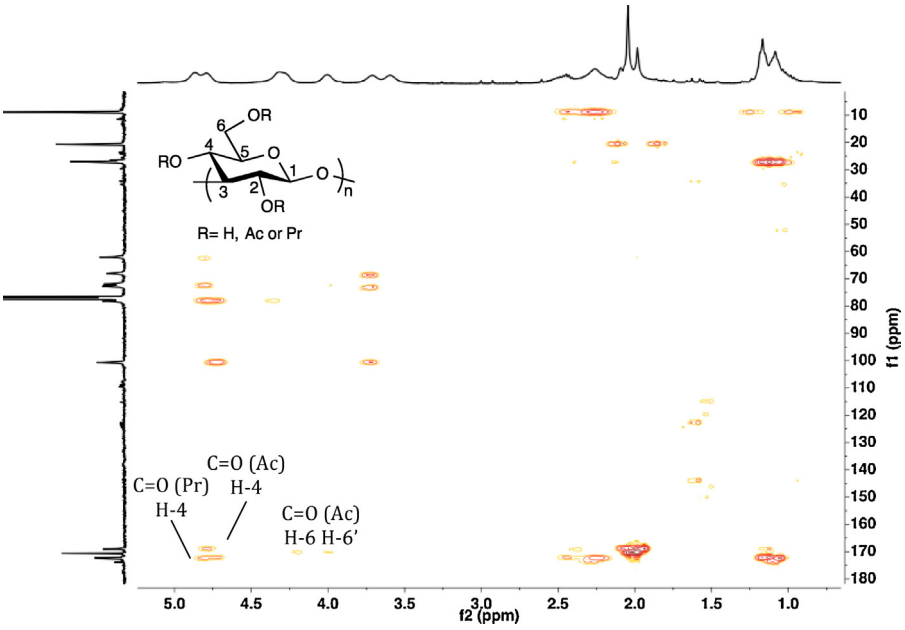

Fig. 4. HMBC NMR spectrum of the product of curdlan triacetate deacylation by TBAF (24 h, THF, 50 °C), after perpropionylation.

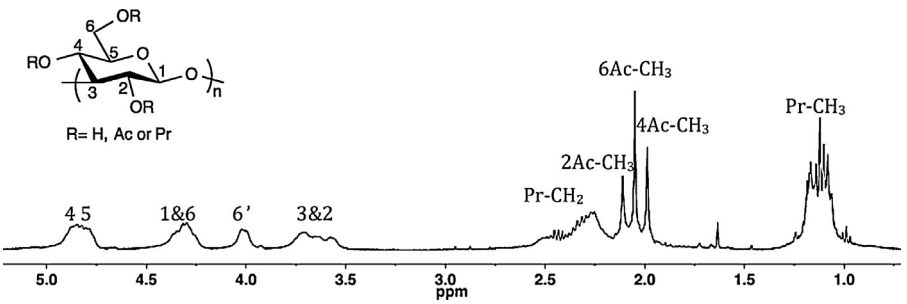

Fig. 5. [1]H NMR spectrum of the product of curdlan triacetate deacylation by TBAOH (1 h, DMSO, RT), after perpropionylation.

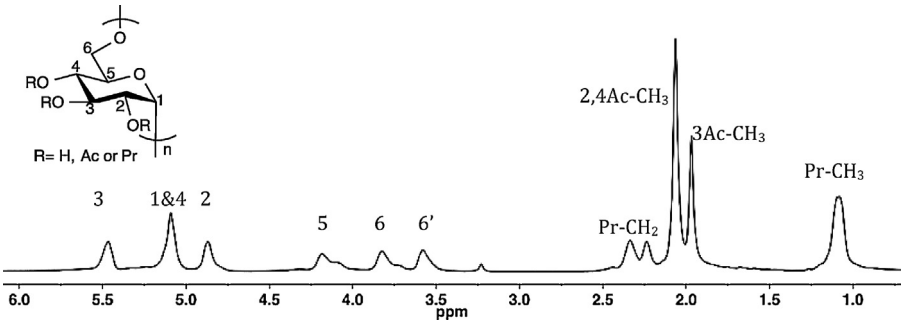

Fig. 6. [1]H NMR spectrum of the product of dextran triacetate deacylation by TBAF (24 h, THF, 50 °C), after perpropionylation.

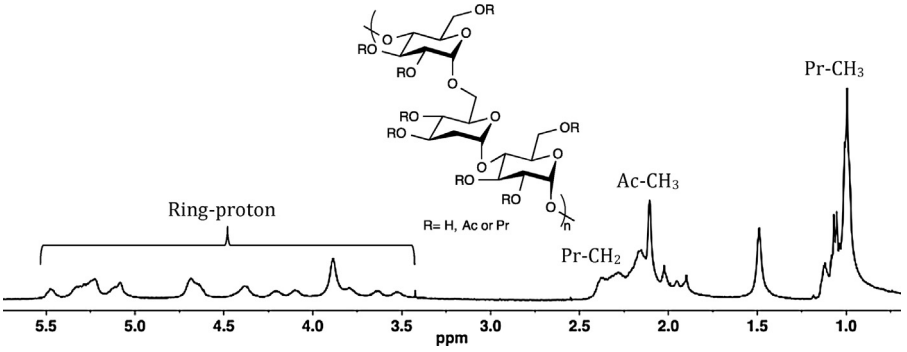

Fig. 7. [1]H NMR spectrum of the product of pullulan triacetate deacylation by TBAF (24 h, DMSO, 50 °C), after perpropionylation.

Table 2
Results of TBAOH deacylation of DTA.[a]

Entry	TBAOH (eq/AGU)	$DS_{Ac(2+4)}$	$DS_{Ac(3)}$	$DS_{Ac(total)}$
1	0	2.00	1.00	3.00
2	1	1.28	0.65	1.93
3	2	0.63	0.32	0.95
4	3	0	0	0

[a] Starting DTA DS 3.0, solvent THF, room temperature, time 1 h.

decreased to the half of above values, with $DS_{Ac(2+4)}$ again approximately twice $DS_{Ac(3)}$, indicating that TBAOH deacylation also led to very similar levels of deacylation of each secondary alcohol acetate. On the other hand, 3 eq TBAOH led to complete deacylation of DTA, as expected. TBAOH deacylation of dextran triacetate is rapid, quantitative, and appears not to be regioselective, consistent with our predictions based on mechanistic understanding and experience with the reaction.

3.4. Deacylation of pullulan triacetate (PTA)

Deacylation of PTA was carried out under the standard TBAF conditions (4 eq TBAF, DMSO, 50 °C, 24 h). This afforded pullulan acetate with DS_{Ac} 1.36. Fig. 7 shows the [1]H NMR spectrum of the PTA deacylation product after perpropionylation. Unfortunately, the acetyl proton resonances for the different positions overlap severely, so it was difficult to completely assign the acetyl protons. Reaction of PTA with 2 eq TBAOH for 24 h at 50 °C gave the resulting pullulan acetate with DS_{Ac} of 1.21; however, in this case also it was difficult to determine regioselectivity by [1]H NMR due to excessive signal overlap.

3.5. Deacylation of glucomannan triacetate (GTA)

Reaction of GTA with 4 eq TBAF for 24 h at 50 °C in DMSO provided successful deacylation, affording glucomannan acetate with DS_{Ac} of 1.10 (Fig. S4). Even though the backbone protons of glucomannan acetate have been completely assigned by a previously published paper (Enomoto-Rogers et al., 2013), it was difficult to determine regioselectivity due to significant signal overlap in these partially substituted glucomannan ester products. The signals at 2.04–2.14 ppm were assigned to C6 acetates of glucose and mannose, C2 acetates of mannose, and C3 acetates of glucose. The resonances between 1.90 and 2.01 ppm were assigned to C2 acetates of glucose and C3 acetates of mannose. Reaction

of GTA with 2 eq TBAOH for 24 h at 50 °C in DMSO also gave the expected deacylation providing glucomannan acetate with DS_{Ac} of 1.00; positional assignment again was precluded by excessive signal overlap.

4. Conclusions

Inspired by discovery of efficient one-step synthetic methods to prepare highly regioselectively substituted cellulose-6-*O*-esters by tetrabutylammonium fluoride- or tetrabutylammonium hydroxide-promoted deacylation, we investigated the effectiveness of these reactions for deacylation of five other polysaccharide triesters, selected for their importance and for the mechanistic and selectivity insights they might provide. Deacylation of amylose tripropionate removed nearly all propionyl groups at the more hindered secondary O-2/3 positions as seen with cellulose esters, showing that the switch from β to α anomeric stereochemistry did not impair regioselectivity. This method for making amylose 6-esters provides nearly perfect regioselectivity and should be useful for the design and synthesis of new regioselectively substituted amylose ester materials. Deacylation of curdlan triacetate was only slightly regioselective, favoring deacylation of the secondary O-2/4 esters over the primary O-6 ester; we attribute this lower regioselectivity to the absence of a vicinal diacetate moiety to provide complexation of the tetrabutylammonium cation and thereby localization of the reactive anion. In deacylation of dextran triacetate, all three positions, O-2/3/6, are esters of secondary alcohols (and constitute two vicinally oriented pairs) and therefore as predicted appeared to undergo the same degree of deacylation without regioselectivity. The complex structures of partially acylated pullulan and glucomannan polysaccharides prevented complete positional resonance assignments in the corresponding NMR spectra, so we were not able to determine the regiochemistry of these deacylations, but did observe the expected total percent deacylations. TBAF did successfully deacylate each of these glycan esters, to approximately the same extent as with cellulose esters under similar conditions. In each case, we also observed that treatment with TBAOH resulted in quantitative removal of a number of equivalents of acyl groups equal to the TBAOH equivalents used.

All of these results are consistent with our previous mechanistic observations and proposals that deacylation at secondary positions differs from that at the primary position, and that regioselectivity is driven by complexation of the tetraalkylammonium cation by vicinal diester groups. Among those five glycan triesters, only amylose tripropionate showed excellent regioselectivity of TBAF and TBAOH deacylations, which is likely due to the fact that it is the only glycan ester examined that has both a vicinal 2,3-ester functionality and a 6-O primary alcohol ester (and which provided product spectra where resonance separation permitted full positional selectivity analysis). Taken together, the results of these explorations of TBAF- and TBAOH-catalyzed deacylations of polysaccharide esters provide confidence about the generality of these reactions in polysaccharide chemistry, and create considerable predictive power to help us understand when we can expect regioselective deacylation, and what that regioselectivity might be, given the structure of the particular polysaccharide ester.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2014.06.053.

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