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Synthesis of curdlan derivatives regioselectively modified at C-6: *O*-(*N*)-Acylated 6-amino-6-deoxycurdlan

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ABSTRACT

There has been growing interest in aminopolysaccharide synthesis over the last two decades due to the critical natural functions of aminopolysaccharides, and their potential in biomedical applications. Regioselective introduction of amino groups into polysaccharide backbones is a challenge. Natural curdlan is a linear β-(1→3)-glucan that is of interest both for its physical properties and its biomedical applications. Aminated curdlan derivatives were synthesized in three steps. First, curdlan was regioselectively brominated at the C-6 position in lithium bromide-N,N-dimethylacetamide (DMAc/LiBr). Second, the bromide of the product 6-bromo-6-deoxycurdlan was displaced by nucleophilic substitution with sodium azide (NaN_3) in dimethyl sulfoxide (DMSO). Third, O-acylated 6-amido-6-deoxycurdlan was produced by a one-pot method. 6-Azido-6-deoxycurdlan was subjected to Staudinger reduction, followed by reaction *in situ* with excess carboxylic anhydride, without isolation of the 6-amino-6-deoxycurdlan intermediate. Regioselectivity and degree of substitution (DS) of these derivatives were confirmed by ^{1}H and ^{13}C NMR spectroscopy, FTIR spectroscopy, and elemental analysis.

1. Introduction

Aminated polysaccharides, in particular glycosaminoglycans, have essential natural functions, largely involving highly specific interactions with proteins. Exemplary functions include cell–cell adhesion (Lokeshwar, Fregien, & Bourguignon, 1994), cell motility (Kim et al., 2008), and prevention of thrombosis (Shriver et al., 2000). These natural functions can be co-opted in disease processes ranging from cancer (Luo, Ziebell, & Prestwich, 2000) to pathogen invasion, for example in Lyme disease (Leong, Robbins, Rosenfeld, Lahiri, & Parveen, 1998) and in maternal malaria (Buffet et al., 1999). Other aminopolysaccharides, especially the natural polymer chitin and its partially N-deacetylated derivative chitosan, have demonstrated significant benefits for biomedical and pharmaceutical applications (Ranaldi, Marigliano, Vespignani, Perozzi, & Sambuy, 2002). As a polycationic compound, chitosan can encapsulate anionic drugs including fatty acids, nucleic acids, and other biological compounds. In nutrition, chitosan is capable of binding fatty acids and bile acids to reduce fat absorption in the intestine, contributing to a reduction of cholesterolemia (Muzzarelli, 2000). In gene delivery, chitosan-based formulations of plasmid DNA encoding the beta-galactosidase reporter gene showed gene expression when transported into the intestines (Köping-Höggård et al., 2001). Additionally, the ability of chitosan to interact with proteins at the tight junctions between enterocytes of the gastrointestinal epithelium, temporarily opening up tight junctions, is of particular importance. Opening tight junctions in transient fashion creates the opportunity for enhanced paracellular permeability of drugs that otherwise have very poor ability to permeate from the gastrointestinal tract into the bloodstream, including important drug candidates like polypeptides (Yeh et al., 2011). Amino groups on the chitosan backbone that are protonated at physiological pH are responsible for these desirable properties; indeed, peralkylation of the amine groups of chitosan to create a permanent positive charge greatly enhances the ability to promote tight junction opening (Thanou, Verhoef, & Junginger, 2001). Despite many advantages, chitin and chitosan formulations have some drawbacks. Complexes of chitosan and nucleic acids have shown poor transfection efficiency (Bowman & Leong, 2006). Moreover, it is difficult to completely remove proteins from chitin when it is extracted from crustacean shells (Raabe, Sachs, & Romano, 2005). It is also not possible to control the sequence of amino groups on the polymer chain during the brutal reaction conditions required for chitin N-deacetylation to produce chitosan.

In an effort to avoid these problems, some researchers have taken the approach of modifying other polysaccharides to mimic the chemical structure of chitosan. The natural, neutral, linear homopolysaccharide, β-(1→3)-glucan (curdlan) is a promising

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candidate. It is produced by the bacterium *Alcaligenes faecalis var.* myxogenes (Harada & Harada, 1996). Curdlan has excellent gelation properties and can form two different types of gels. One is a thermally reversible low-set gel formed at temperatures close to 55 °C while the other is a nonreversible high-set gel formed at temperatures greater than 80 °C (McIntosh, Stone, & Stanisich, 2005). This ability to gel has been utilized in the food industry, where curdlan is employed as a biothickening/gelation agent (Lee, 2002) and a fat/meat substitute (McIntosh et al., 2005), and mimics the rheological properties of sauces and dairy products (Funami, Yada, & Nakao, 1998), as well as in other applications. Sulfated curdlan derivatives have also been explored as anti-HIV agents (Borjihan, Zhong, Baigude, Nakashima, & Uryu, 2003; Gao et al., 1997; Osawa et al., 1993). One approach to aminocurdlans has been the application of Huisgen azide-alkyne 'click chemistry' (Huisgen, 1963) to attach amine-containing side chains to the curdlan backbone (Hasegawa, Umeda, Numata, Li, et al., 2006). However, there are very few reports in the literature of attempts to replace the hydroxyl groups of curdlan with amine substituents. With its β-1→3 linkages, the curdlan anhydroglucose unit (AGU) has three hydroxyl groups, a primary hydroxyl at C-6 and two secondary hydroxyls at C-2 and C-4. The primary 6-OH is the most reactive hydroxyl group in reactions where it plays the role of nucleophile.

There have been a number of studies detailing regioselective C-6 amination of other polysaccharides including cellulose (Fox & Edgar, 2012; Heinze, Koschella, Brackhagen, Engelhardt, & Nachtkamp, 2006; Liu & Baumann, 2002; Matsui, Ishikawa, Kamitakahara, Takano, & Nakatsubo, 2005) and amylose (Cimecioglu, Ball, Kaplan, & Huang, 1994). The precursors for these reactions are typically 6-azido polysaccharides. Early reports demonstrated a one step reaction to form azido derivatives of cyclodextrin (Boger, Corcoran, & Lehn, 1978), amylose and pullulan (Cimecioglu, Ball, Huang, & Kaplan, 1997) using carbon tetrabromide (CBr_4), lithium azide (LiN_3) and triphenylphosphine (Ph_3P) in dimethylformamide (DMF). However, inadequate control over the degree of substitution as well as side reactions limited the utility of those reactions. Thus, separating the bromination from the azidation step is essential. Hasegawa, Umeda, Numata, Fujisawa, et al. (2006) and Hasegawa, Umeda, Numata, Li, et al. (2006) synthesized 6-bromo-6-deoxy-curdlan in DMF/LiCl with CBr_4. The long reaction time (>24 h) and partial substitution at C-6 of chloride from LiCl were major concerns. Furuhata, Koganei, Chang, Aoki, and Sakamoto (1992) and Tseng, Furuhata, and Sakamoto (1995) later successfully used N-bromosuccinimide (NBS) and Ph_3P in DMAc/LiBr to accomplish regioselective bromination of cellulose and chitin, respectively.

Herein, we report our attempts to achieve regioselective bromination of curdlan at C-6 using NBS and Ph_3P in DMAc/LiBr under homogeneous conditions. Our intention was to use this 6-bromo-6-deoxy derivative as a substrate for azide (NaN_3) displacement (Scriven & Turnbull, 1988) to introduce nitrogen-containing functionality to C-6. We anticipated that Staudinger reduction of this azide, optionally with controlled N,O-acylation, might serve as an entry to new families of 6-amino-6-deoxycurdlan derivatives, which could be of significant interest for biomedical applications and for studying the specificity of their interactions with proteins. We report herein the results of these studies.

2. Experimental

2.1. Materials

β-(1→3)-Glucan (curdlan, $(-C_6H_{10}O_5-)_n$) was obtained from Wako Chemicals and dried under vacuum at 40 °C overnight prior to use. LiBr (laboratory grade, Fisher) and NaN_3 (99%, Alfa Aesar) were dried under vacuum at 125 °C. N-Bromosuccinimide (99%, Acros) was recrystallized from boiling water and dried for two days under reduced pressure over anhydrous calcium chloride. Triphenylphosphine (Ph_3P, 99%, Acros), acetic anhydride (Ac_2O, 99+%, Acros) and propionic anhydride (Pr_2O, 97%, Sigma–Aldrich) were used as received. DMAc (reagent grade, Fisher) and DMSO (HPLC grade, Fisher) were kept over 4 Å molecular sieves and stored under dry nitrogen until used. Acetone (HPLC grade, Fisher), methanol (HPLC grade, Fisher) and ethanol (HPLC grade, Fisher) were used as received.

2.2. Measurements

^{13}C NMR spectra were acquired on INOVA 400 or Bruker Avance 500 spectrometers with a minimum of 10,000 scans at 50 °C. Samples were analyzed as solutions in $CDCl_3$ or DMSO-d_6 (ca. 10 mg/mL) in standard 5 mm o.d. tubes. Chemical shifts are reported relative to the solvent peaks. Infrared spectroscopic analyses of samples as pressed KBr pellets were performed on a Thermo Electron Nicolet 8700 instrument. Elemental analyses (EA) to determine carbon, nitrogen and bromine contents were performed by Micro Analysis Inc. using a Perkin Elmer 2400 II analyzer. Carbon and nitrogen contents were determined by flask combustion followed by ion chromatography and bromine content was measured with a thermal conductivity detector.

2.3. Methods

2.3.1. Dissolution of curdlan in DMAc/LiBr

The method was adapted from a method previously reported for cellulose dissolution (Edgar, Arnold, Blount, Lawniczak, & Lowman, 1995). Dried curdlan (4.00 g, 24.7 mmol AGU) was weighed into a 250 mL, three-necked round-bottom flask. The flask was fitted with a nitrogen inlet, short-path distillation unit and overhead stirrer. Next, 150 mL DMAc was added to the flask, and the contents were stirred. The flask was flushed with dry nitrogen and heated in an oil bath at 150 °C for 26 min. LiBr (36.00 g, 42.4 mmol) was added to the flask and the mixture was heated at 170 °C for 8 min. The distillate (40 mL) was collected under a stream of nitrogen at 170 °C. The reaction mixture was allowed to cool to room temperature while being stirred continuously. Curdlan dissolved within 3 h to form a transparent solution.

2.3.2. Regioselective bromination of curdlan

Ph_3P (25.96 g, 4 eq per AGU) was dissolved in 50 mL of dry DMAc. A second solution of NBS (17.58 g, 4 eq per AGU) was prepared in an additional 50 mL of dry DMAc. The Ph_3P solution was added dropwise via a liquid addition funnel to a solution of 4.00 g curdlan in DMAc/LiBr, prepared as described above. Subsequently, the NBS solution was added in similar fashion. The reaction solution was then heated at 70 °C for 1 h. The reaction mixture was added slowly to 1 L of a 50:50 mixture of methanol and deionized water. The reaction mixture was held overnight at room temperature. The mixture was then filtered to recover the precipitate. The isolated product was re-dissolved in DMSO and re-precipitated in ethanol twice. The sample was dried under vacuum at 40 °C overnight to yield 6-bromo-6-deoxycurdlan. IR (KBr): ν 3445 (O—H), 2906 (C—H), 541 (C—Br). ^{13}C NMR (DMSO-d_6): δ 103.2 (C-1), 84.9 (C-3), 74.4 (C-5), 73.6 (C-2), 70.1 (C-4), 34.6 (C-6-Br) ppm. Elemental analysis: Br % = 28.41 (Theoretical Br % 35.53 if DS(Br) = 1). Yield: 4.75 g (86%).

2.3.3. Synthesis of 6-azido-6-deoxy-(2,4-di-O-acyl-)curdlan

Dry 6-bromo-6-deoxycurdlan (1.00 g, 4.44 mmol) was dissolved in 25 mL of anhydrous DMSO in a 100 mL flask. Then, NaN_3 (1.44 g, 5 eq per AGU) was added to the flask and dissolved. The solution was heated to 80 °C and stirred for 24 h under nitrogen. The product was

isolated by pouring the reaction mixture into 300 mL of deionized water and the precipitate was collected by filtration. The product was re-dissolved in acetone and then re-precipitated in deionized water, followed by filtration. The sample was dried under vacuum at 40 °C overnight to yield 6-azido-6-deoxycurdlan. IR (KBr): ν 3460 (O—H), 2916 (C—H), 2109 (N_3). ^{13}C NMR (DMSO-d_6): δ 103.4 (C-1), 84.9 (C-3), 74.9 (C-5), 73.9 (C-2), 69.4 (C-4), 51.7 (C-6-N_3) ppm. Elemental analysis: *N*% = 18.84 (Theoretical *N*% = 22.45 if DS(N_3) = 1). Yield: 0.76 g (92%).

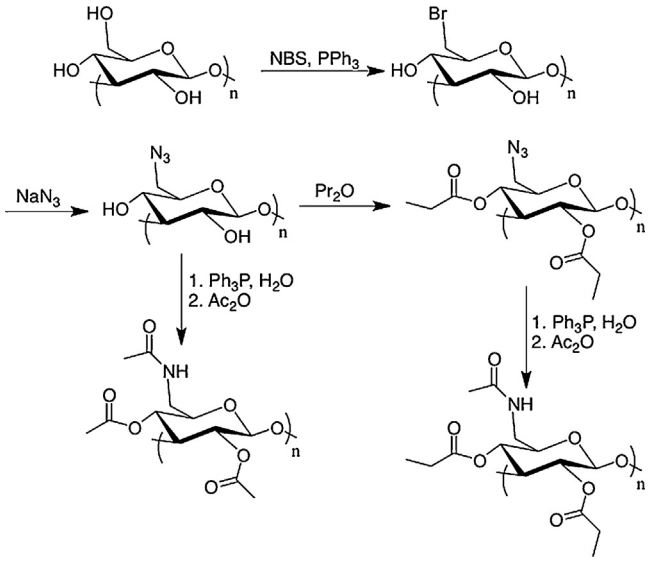
Under nitrogen, dry 6-azido-6-deoxycurdlan (1.00 g, 5.34 mmol) and 4-dimethylaminopyridine (DMAP, 20 mg) were weighed into a 50 mL round-bottom flask. Pyridine (4.3 mL, 10 eq per AGU) and 10 eq of a carboxylic anhydride (acetic or propionic anhydride) were then added dropwise to the solution. The mixture was reacted at 80 °C for 24 h under nitrogen. The homogeneous mixture was then added slowly to 200 mL deionized water. The crude product was collected by filtration, and then re-dissolved in 10 mL chloroform. This solution was added slowly with rapid stirring to 200 mL of ethanol. After filtration and washing with ethanol and water several times, the sample was dried under vacuum at 40 °C overnight to yield 6-azido-6-deoxy-2,4-di-*O*-acyl-curdlan. ^{13}C NMR (DMSO-d_6): δ 169.6 (C=O), 99.4 (C-1), 78.0 (C-3), 72.1 (C-5), 71.1 (C-2), 68.5 (C-4), 50.5 (C-6-N_3), 20.6 (CH_3) ppm. Yield: 1.40 g (6-azido-6-deoxy-2,4-di-*O*-acetyl-curdlan, 97%), 1.48 g (6-azido-6-deoxy-2,4-di-*O*-propionyl-curdlan, 93%).

2.3.4. Synthesis of (O-acylated-) 6-amino-6-deoxycurdlan

In a representative example, 0.20 g 6-azido-6-deoxy-(2,4-di-*O*-acyl-)curdlan was dissolved in 20 mL of DMAc, followed by the dropwise addition of 0.10 mL of deionized water. Ph_3P (0.56 g for 6-azido-6-deoxy-curdlan, 2 eq per AGU; 0.39 g for 6-azido-6-deoxy-2,4-di-*O*-acetyl-curdlan, 2 eq per AGU) was then added to the 50 mL round-bottom flask and the reaction was run under ambient conditions in a flask with a stopper to prevent solvent loss. After allowing the reaction to proceed for 12 h, the solution was transferred to 3500 g/mol MWCO dialysis tubing that was then placed in a large beaker containing ethanol. As the dialysis of the reaction solution progressed, a light brown precipitate slowly formed within the tubing. After three days of dialysis, the contents of the tubing were removed. The precipitate was isolated by filtration and then dried under vacuum at 40 °C overnight to yield 6-amino-6-deoxy-(2,4-di-*O*-acyl-)curdlan. IR (KBr): ν 3450 (O—H), 2945 (C—H), 2590 (N—H). Yield: 0.11 g (6-amino-6-deoxy-curdlan, 64%), 0.11 g (6-amino-6-deoxy-2,4-di-*O*-acetyl-curdlan, 61%).

2.3.5. Synthesis of 6-amido-6-deoxy-2,4-di-O-acyl-curdlan

In a 100 mL round-bottom flask, 6-azido-6-deoxycurdlan (0.50 g, 2.67 mmol) was dissolved in 50 mL DMAc under dry nitrogen, followed by the dropwise addition of 0.25 mL of deionized water. As Ph_3P (1.41 g, 2 eq per AGU) was added to the flask, the reaction began as evidenced by solution color change from dark brown to light brown and appearance of some small bubbles. The reaction was run under ambient conditions in a flask with a stopper to prevent solvent loss. The reaction remained homogeneous for 12 h. Then, 50 eq per AGU of a carboxylic anhydride was added to the flask followed by 20 mg of DMAP and 5 mL of pyridine. The solution was stirred for 24 h at 80 °C under dry nitrogen. The solution was transferred to 3500 g/mol MWCO dialysis tubing (prewet with water) that was placed in a large beaker containing deionized water. As the dialysis of the reaction solution progressed, a precipitate slowly formed within the tubing. After one day of dialysis, the contents of the tubing were removed, and the precipitate was isolated by filtration. The precipitate was further purified by Soxhlet extraction with ethanol for 1 day, and then dried in a vacuum oven at 40 °C overnight.



Scheme 1. Conversion of curdlan to 6-amido-6-deoxy-2,4-di-*O*-acyl-curdlan.

3. Results and discussion

3.1. Regioselective bromination of curdlan

DMAc/LiBr (Furuhata et al., 1992; Tseng et al., 1995) was employed as the solvent system for curdlan dissolution and reaction, due to its proven ability to dissolve polysaccharides, interrupt polysaccharide H-bonding, and its compatibility with a wide variety of synthetic methods. We initially attempted to brominate curdlan in DMSO since it is a solvent for curdlan, NBS and Ph_3P. However, after adding NBS and Ph_3P, the reaction was exothermic and difficult to control, resulting in foaming out of the flask. Clearly the rather reactive DMSO is not a suitable solvent for this transformation. Other investigators (Dalton, Dutta, & Jones, 1968; Dalton, Rodebaugh, & Jefford, 1972) have reported exotherms upon DMSO/NBS mixing, although to our knowledge no detailed mechanistic studies have been done. The powerful mixed solvent system (DMAc/LiBr) is known to be effective for dissolution and chemical reactions of the recalcitrant cellulose; the use of LiBr rather than LiCl prevents halogen exchange in the bromopolysaccharide product. In the event, curdlan did dissolve in this solvent system successfully and it was suitable for the further reactions we had in mind.

Curdlan bromination (Scheme 1) with NBS and Ph_3P took place regioselectively at C-6 in homogeneous solution in DMAc/LiBr by procedures analogous to those previously used to brominate cellulose (Furuhata et al., 1992) and chitin (Tseng et al., 1995). It is believed that substitution of hydroxyl by bromide involves two consecutive S_N2 reactions, thereby ensuring virtually complete selectivity for the less hindered and more nucleophilic 6-OH. First, PPh_3 is brominated by NBS to form a bromotriphenylphosphonium ion, followed by S_N2 displacement of Br- from this intermediate by the 6-OH group to produce an alkoxyphosphonium salt intermediate (Fox & Edgar, 2011). Backside attack by bromide anion on C-6 displaces triphenylphosphine oxide as leaving group, affording 6-bromo-6-deoxycurdlan.

Spectroscopic analysis of the 6-bromo-6-deoxycurdlan product confirmed that the reaction was highly chemo- and regioselective. The FTIR spectrum (Fig. 1) shows a broad hydroxyl stretching absorption near 3500 cm^{-1}, and C—H stretching absorption around 2900 cm^{-1} from the curdlan backbone. The relatively weak peak near 550 cm^{-1} is attributed to the C—Br stretching absorption. More conclusive evidence of conversion to the 6-bromo derivative is provided by the ^{13}C NMR spectrum (Fig. 2). Distinct, single peaks

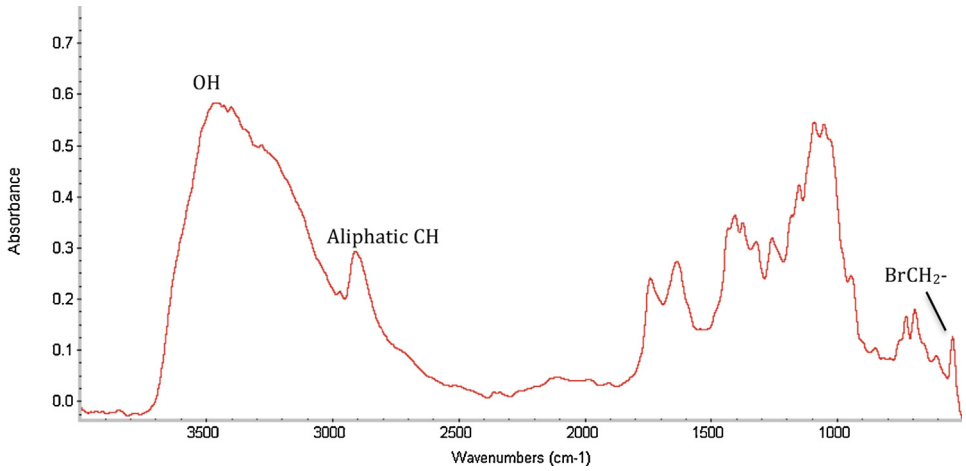


Fig. 1. FTIR spectrum of 6-bromo-6-deoxycurdlan (A).

for C-1 to C-5 of the curdlan backbone are present between 65 and 105 ppm (Hasegawa, Umeda, Numata, Fujisawa, et al., 2006; Hasegawa, Umeda, Numata, Li, et al., 2006). The strong upfield chemical shift for C-6 upon substitution by bromine to 34 ppm is diagnostic for the postulated bromination. No peak is observed at 60 ppm where the original C-6-OH of pure curdlan would resonate, indicating essentially complete conversion. Previous curdlan brominations (Hasegawa, Umeda, Numata, Fujisawa, et al., 2006; Hasegawa, Umeda, Numata, Li, et al., 2006) were carried out in DMF/LiCl using CBr_4, but DS values were not reported. The DS of bromide was calculated to be 0.80 by elemental analysis. This, however, is almost certainly a significant underestimate, due to the presence of residual triphenylphosphine and triphenylphosphine oxide that are very difficult to remove quantitatively (vide infra). All NMR data on this and subsequent intermediates and products indicate that bromination was complete and that actual 6-Br DS approaches 1.0.

3.2. 6-Azido-6-deoxycurdlan derivatives by azide displacement

Hasegawa et al. have shown that 6-bromo-6-deoxycurdlan is an effective intermediate for subsequent S_N2 displacement by azide. These researchers carried out this substitution reaction by reacting the 6-bromo product with NaN_3 in DMSO at 80 °C for 36 h (Hasegawa, Umeda, Numata, Fujisawa, et al., 2006; Hasegawa, Umeda, Numata, Li, et al., 2006). We first followed this procedure to obtain the azido product and confirmed its chemical structure by FTIR (Fig. 3a) and ^{13}C NMR spectra (Fig. 4). The strong $-N_3$ absorption at 2108 cm^{-1} indicates successful incorporation of azide. In the ^{13}C NMR spectrum the C-6 chemical shift appears at 51 ppm, shifted significantly downfield from the C-6 of the bromodeoxy starting material (34 ppm). Since different substituents at C-2 are known to cause multiplicity of signals for the anomeric carbons (C-1), the absence of such multiplicity here is additional evidence for the selectivity of the bromination/azidation sequence for substitution at C-6. Moreover, no peak at 60 ppm for C-6-OH was observed, indicating that no hydrolysis at C-6 occurred during azide substitution. There is also no trace of the brominated carbon at 34 ppm.

In order to figure out the optimal reaction time for this azide displacement, we monitored the process by removal of aliquots of the reaction solution at predetermined lengths of time. Each product was isolated for elemental analysis and the results are shown in Table 1. Since the apparent DS of the azide levels off around 0.89 after 1 h, we shortened this displacement reaction time from 36 h to 1 h. Product elemental analysis indicated no Br content after 1 h, indicating complete bromide displacement. The ^{13}C NMR spectrum of the 1 h product (not shown) also displays only a single peak for C-6-N_3 at 51 ppm, confirming the clean and rapid nature of this reaction.

Conversion of pure 6-azido-6-deoxycurdlan to its peracylated derivatives was simple and straightforward. 6-Azido-6-deoxycurdlan was reacted with acetic or propionic anhydride (pyridine, DMAP, 80 °C, 24 h) to fully esterify the free 2- and 4-hydroxyls. Product structures were confirmed by ^{13}C NMR spectroscopy, in which the 2,4-diacetate carbonyl peaks appear around 170 ppm. In the ^{1}H NMR spectrum of the 2,4-diacetate, there are two peaks around 2.1 ppm for the acetyl methyl groups. The FTIR spectrum shows a strong ester carbonyl stretch around 1750 cm^{-1}. These derivatives are effective precursors

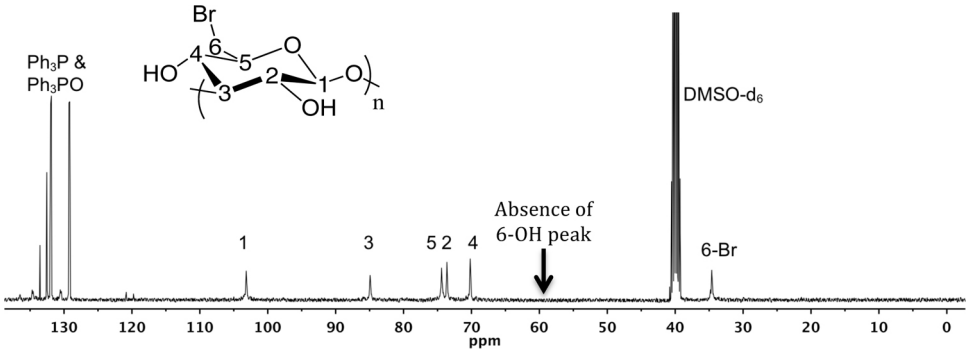


Fig. 2. ^{13}C NMR spectrum of 6-bromo-6-deoxycurdlan (A).

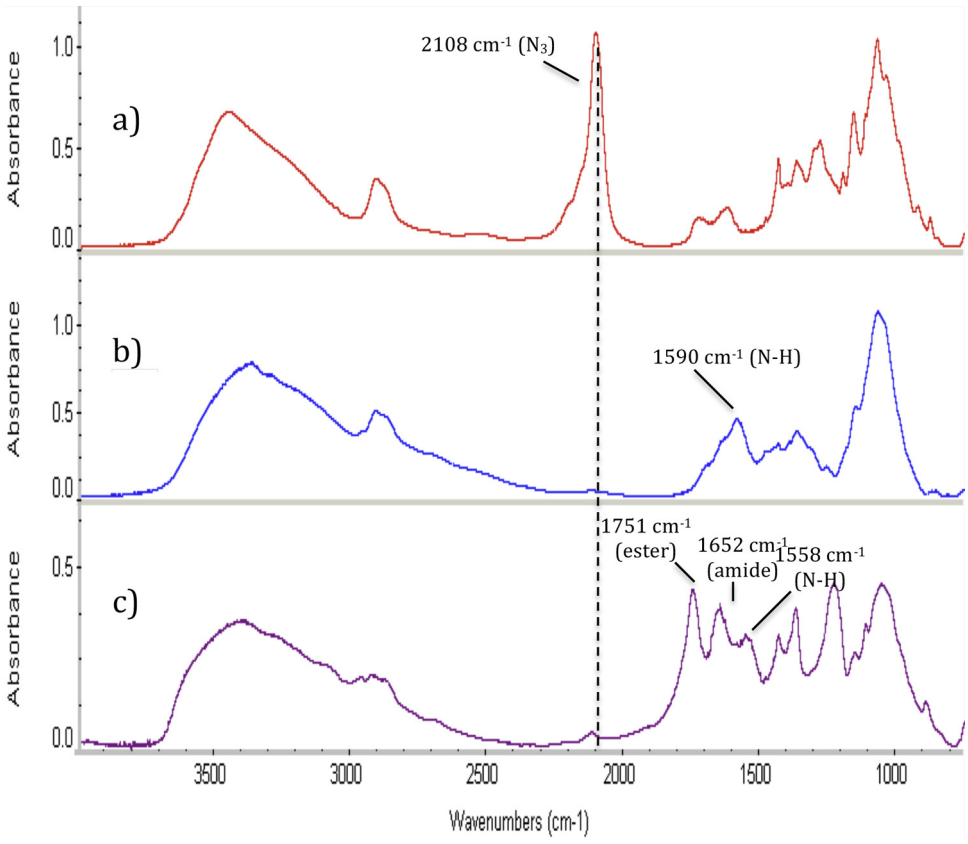


Fig. 3. FTIR spectra of (a) 6-azido-6-deoxycurdlan, (b) 6-amino-6-deoxycurdlan and (C) 6-amino-6-deoxy-2,4-di-*O*-acetyl-curdlan synthesized by Staudinger reduction.

Table 1
Kinetics of azide displacement on 6-bromo-6-deoxycurdlan in DMSO at 80 °C[a].

Reaction time (h)	0.50	0.75	1	2	3	4	5	10	24	36
N_3 wt%	12.47	16.87	19.93	19.93	19.94	19.94	19.12	19.04	18.84	19.07
DS (N_3)	0.555	0.751	0.887	0.887	0.888	0.888	0.851	0.847	0.838	0.846

[a] Measured by elemental analysis.

for the subsequent synthesis of 6-amido-6-deoxycurdlan-2,4-*O*-esters, in which the *N*-acyl group differs from the ester acyl groups.

3.3. Synthesis of (*O*-acylated-) 6-amino-6-deoxycurdlan and 6-amido-6-deoxy-2,4-di-*O*-acyl-curdlans.

Our first approach to aminocurdlan derivatives of interest was to synthesize (*O*-acylated-) 6-amino-6-deoxycurdlan, then carry out N-acylation (potentially with a different acyl group) to obtain 6-amido-6-deoxy-2,4-di-*O*-acyl-curdlan. A Staudinger reduction (PPh_3, H_2O, DMSO, room temperature) was used to reduce the azide to amine due to the mild conditions and high selectivity permitted by this approach (Scriven & Turnbull, 1988). Our previous work indicated that Staudinger reduction of polysaccharide azides to amines is sufficiently mild to preserve ester bonds against reduction (Fox & Edgar, 2012). The azide reduction begins with formation of a phosphazide by reaction between 6-azido-6-deoxy-(2,4-di-*O*-acyl-)curdlan and Ph_3P. An iminophosphorane forms after the loss of nitrogen gas, followed by aqueous hydrolysis to produce,

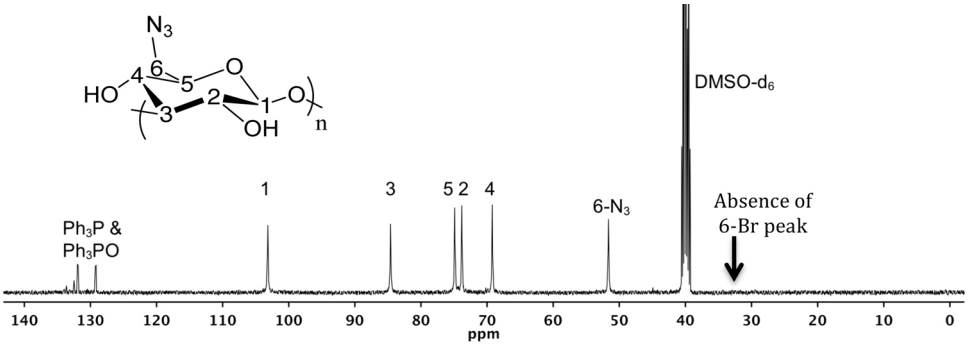


Fig. 4. ^{13}C NMR spectrum of 6-azido-6-deoxycurdlan (B).

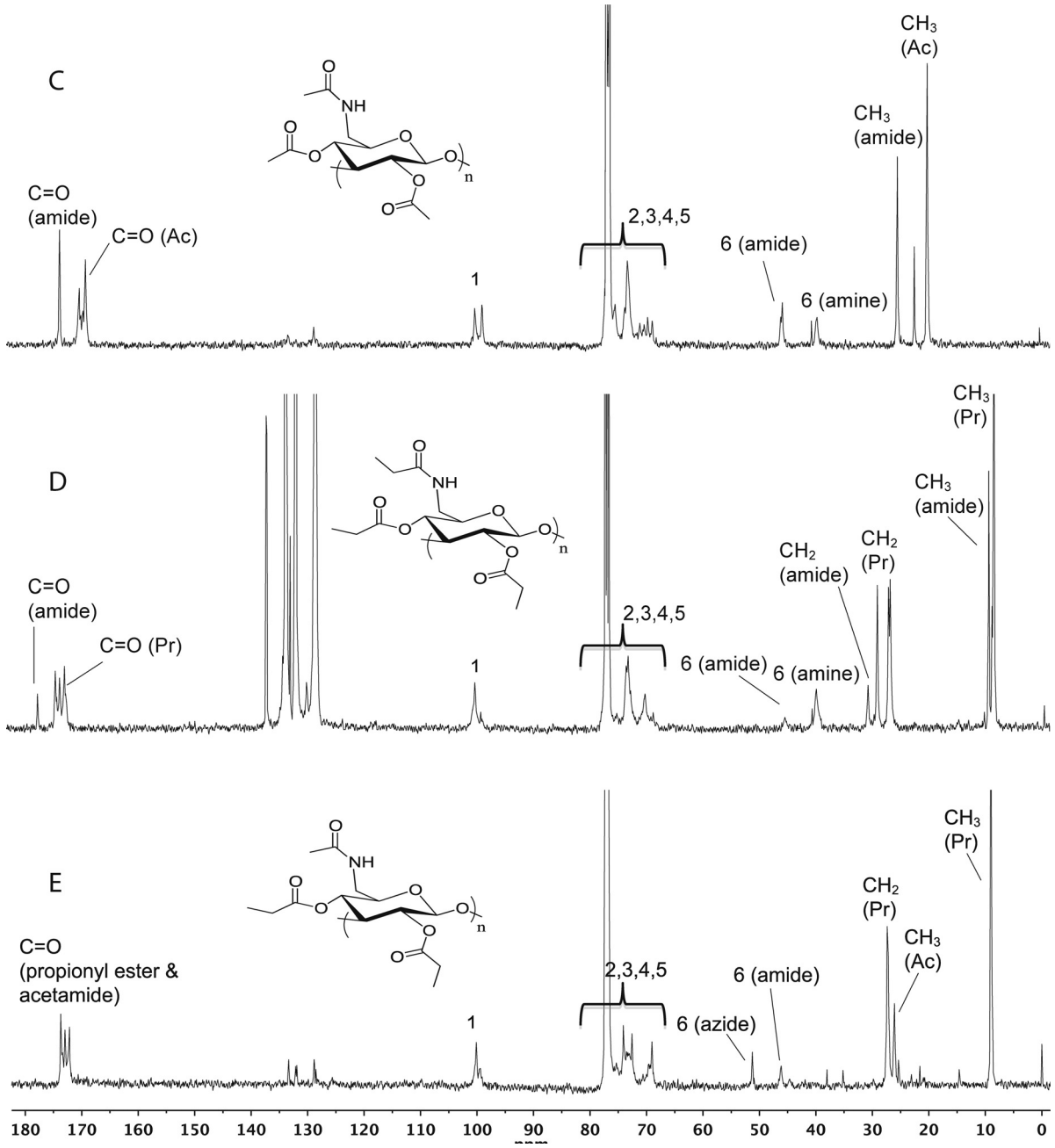


Fig. 5. ^{13}C NMR spectra of 6-acetamido-6-deoxy-2,4-di-*O*-acetyl-curdlan (C), 6-propionamido-6-deoxy-2,4-di-*O*-propionyl-curdlan (D), and 6-acetamido-6-deoxy-2,4-*O*-propionyl-curdlan (E).

in this case, 6-amino-6-deoxy-(2,4-di-*O*-acyl-)curdlan and triphenylphosphine oxide (Ph_3PO) (Fox & Edgar, 2012).

After overnight Staudinger reduction at room temperature, the reaction mixture was dialyzed against ethanol for at least three days in order to obtain the 6-amino-6-deoxy-(2,4-di-*O*-acyl-)curdlan. Interestingly, once isolated, the products were found to be insoluble in any common NMR solvents including water, DMSO, chloroform and acetone, preventing characterization by solution NMR. Fig. 3b and c shows the FTIR spectra of 6-amino-6-deoxycurdlan and 6-amino-6-deoxy-2,4-di-*O*-acetyl-curdlan, respectively, prepared by such Staudinger reduction. Small residual azide absorptions are evident at 2108 cm^{-1}, though peak intensity is greatly reduced (compare with Fig. 3a). Absorptions at 1590 and 1558 cm^{-1} are assigned to N—H bending of the free amine, confirming azide reduction by Ph_3P. An absorption band at 1652 cm^{-1}, typically associated with amide carbonyl stretches, is present in 6-amino-6-deoxy-2,4-di-*O*-acetyl-curdlan but not in 6-amino-6-deoxycurdlan. This is probably the result of partial amide formation, resulting from the nucleophilic attack of the nitrogen of the iminophosphorane upon the carbonyl carbons of the 2, 4-*O*-ester groups present on the curdlan chain (Fox & Edgar, 2012). The poor solubility of these isolated amino products is likely due to intermolecular hydrogen bonding between the 6-amino groups, and would be limiting with regard to their further application as drug or gene delivery agents in biomedical fields. Therefore, we designed the synthesis of amido-derivatized curdlans with enhanced solubility.

To produce amido curdlan esters with identical *N*-acyl and *O*-acyl groups, we directly added excess carboxylic anhydride to

Table 2
^{13}C NMR chemical shift assignments for acetyl and propionyl groups in 6-amido-6-deoxycurdlan esters[a].

Acetyl		Propionyl	
Carbon	δ (ppm)	Carbon	δ (ppm)
CH_3 (ester)	20.6	CH_3 (ester)	27.3
CH_3 (amide)	26.2	CH_3 (amide)	31.3
C=O (ester)	170.1	CH_2 (ester)	8.9
C=O (amide)	173.6	CH_2 (amide)	9.8
		C=O (ester)	173.6
		C=O (amide)	177.6

[a] All spectra were determined using $CDCl_3$ solutions.

the reaction mixture of 6-azido-6-deoxycurdlan after Ph_3P reduction without isolating the intermediate 6-amino-6-deoxycurdlan. In order to target derivatives in which the *N*-acyl group differed from the *O*-acyl groups, esterified 6-azido curdlans were used as starting materials (Scheme 1). They were first reduced through Staudinger reaction and then a carboxylic anhydride (with acyl moieties distinct from those of the *O*-acyls) was added to form an amide selectively while preserving the 2,4-*O*-ester groups. All amido products were soluble in $CDCl_3$, permitting ready acquisition of solution ^{1}H and ^{13}C NMR spectra (Fig. 5).

In each case, the chemical shift for C-6 with a pendant amide group is 46 ppm. An aminated C-6 peak at 40 ppm shows up in C and D while a residual C-6-azide peak at 51 ppm appears in spectrum E, indicating incomplete *N*-acylation and Staudinger reduction, respectively. Compared with those of ester substituents (acetate or propionate), the aliphatic and carbonyl carbons are shifted downfield in the amide groups (acetamide or propionamide). Table 2 summarizes the chemical shifts for carbons in esters and amides of 6-amido-6-deoxy-curdlan esters. Despite washing the products with excess water and ethanol several times, there are some small peaks evident in the range of 128–135 ppm, attributed to residual aryl phosphorus reagent and by-product (Ph_3P & Ph_3PO). Soxhlet extraction was the most effective method (vs. for example reprecipitation) for further purification of the amidocurdlan products, significantly reducing (but not entirely eliminating) the intensity of aromatic peaks in ^{13}C NMR spectra.

4. Conclusions

We have developed useful methods for synthesis of a variety of regioselectively substituted aminocurdlan derivatives. Curdlan dissolution in DMAc/LiBr has been demonstrated, along with regioselective, safe bromination with NBS and Ph_3P in DMAc/LiBr to obtain 6-bromo-6-deoxycurdlan, with high DS(Br) and no evidence of side products or reaction incompletion. We have determined the kinetics of the subsequent azide displacement reaction in DMSO, establishing that it is virtually complete within 1 h, again with no spectroscopic evidence of incompletion or side reactions. We have demonstrated multiple routes for conversion of the azide to amine or amides, creating the option of appending the same acyl group to form both ester and amide groups, or alternatively different *N*- and *O*-acyl groups forming dissimilar 6-amide and 2,4-diester groups. The mild Staudinger reaction is useful for selective reduction of azide to amine in the presence of ester groups, and is also tolerant of the presence of acylating reagent in situ for capturing the iminophosphorane intermediate as it is generated, in order to avoid migration of acyl groups from the ester moieties to the iminophosphorane nitrogen. We found that amide products have much improved solubility vs. 6-amino-6-deoxycurdlan or its *O*-esters. It is interesting to note that 6-aminopolysaccharide derivatives generally appear to have poor solubility in both water and organic solvents; we have made this observation in our lab for derivatives of curdlan, cellulose (Fox & Edgar, 2012), and pullulan (Pereira, 2013). It is necessary to further derivatize these 6-aminopolysaccharides in order to obtain solubility and thereby processability; herein we present *N*- and *O*-acylation as methods for achieving this goal, and will report others in future publications. The synthetic methods we report here open doors to a wide variety of potentially useful aminocurdlan and amidocurdlan polymers. The biomedical properties of these and related curdlan derivatives are of great interest to us and will be the topics of further study in our laboratory.

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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.2014.01.045.

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