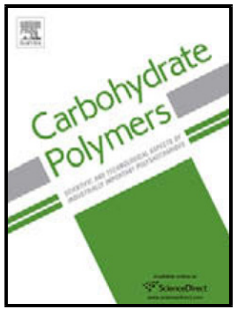

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Efficient Synthesis of Secondary Amines by Reductive Amination of Curdlan Staudinger Ylides

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Highlights:

- Regioselective synthesis of curdlan derivatives
- Synthesis of curdlan 6-imines by reductive amination
- Synthesis of curdlan secondary amines by reductive amination
- Completes synthetic arsenal for 6-amino/amido curdlans

Abstract

Staudinger-related reactions between azides and phosphines are important in organic chemistry due to their chemoselectivity, high efficiency, and mild reaction conditions. Staudinger reduction of azides affords highly reactive iminophosphorane ylides; the reactivity of the negatively charged ylide nitrogen atom has not previously been fully explored in polysaccharide chemistry. Curdlan, a natural, biocompatible and bioactive β-1,3-glucan with low toxicity, has remarkable potential in biomedical and pharmaceutical applications. Herein we describe a new method for preparation of regioselectively iminated/aminated curdlan derivatives via a Staudinger ylide. 6-Azido-6-deoxy-2,4-di-*O*-acyl-curdlan was treated with triphenylphosphine to generate the highly nucleophilic iminophosphorane intermediate which afforded: i) 6-imino curdlans by reaction with several aromatic aldehydes, and ii) 6-monoalkylamino curdlans by reductive amination using these aldehydes

and $NaBH_3CN$. This new chemistry combined with our previous results makes available valuable primary, secondary, and tertiary amines, quaternary ammonio derivatives, and amides, all with complete C-6 regioselectivity for the N-substitution.

Keywords: Reductive amination; curdlan; secondary amine; Staudinger; ylide; polysaccharide derivative

1. Introduction

Natural polysaccharides are remarkably abundant, diverse materials that have many important functions in living organisms. These sustainable polymers are underutilized, with only a few being used to create functional derivatives to serve society (albeit in important ways). In order to effectively utilize polysaccharides, we need to understand the fundamental relationship between structure and properties, and we need better methods for selective modification of natural polysaccharides. Curdlan, an extracellular bacterial polysaccharide, is of significant interest due to its valuable rheological properties and inherent bioactivity. The simple homopolymeric, unbranched and uncharged structure of the (1,3)-β-D-glucan curdlan can be elaborated using a range of chemical modifications, such as esterification (Marubayashi, Yukinaka, Enomoto-Rogers, Takemura, & Iwata, 2014), carboxymethylation (Jin, Zhang, Yin, & Nishinari, 2006), phosphorylation (Suflet, Nicolescu, Popescu, & Chitanu, 2011), sulfation (Osawa et al., 1993; Yoshida et al., 1990) and TEMPO oxidization (Delattre et al., 2009; Tamura, Hirota, Saito, & Isogai, 2010). Our laboratory has developed a series of regioselective C-6 modifications of curdlan to synthesize 6-deoxy-6-(bromo/azido/amino/amido/ammonium) derivatives (Zhang & Edgar, 2014, 2015; Zhang, Liu, & Edgar, 2016) that are promising candidates for biomedical and pharmaceutical applications. We have observed that the iminophosphorane intermediate generated during Staudinger reduction of 6-azido-6-deoxycurdlan is highly nucleophilic, in accord with the previous work of Bertozzi (Saxon, Armstrong, & Bertozzi, 2000; Saxon & Bertozzi, 2000) and others. 6-Azido-6-deoxycurdlan is an

efficient ylide precursor that upon treatment with triphenylphosphine at ambient temperature forms a phosphazide, which in turn loses nitrogen gas to form the desired iminophosphorane.

Iminophosphoranes are organic compounds of general composition $R_3P{=}NR$ that possess a highly polarized P=N bond and are best described as resonance hybrids of the two extreme forms A and B (**Fig. S1**) (García-Álvarez, García-Garrido, & Cadierno, 2014). Staudinger reaction of a phosphine (R_3P:) with an organic azide is by far the most widely used method to synthesize iminophosphoranes. Although Staudinger and Meyers (Staudinger & Meyer, 1919) prepared the first aza-Wittig reagent $Ph_3P{=}NPh$ in 1919, the chemistry of iminophosphoranes was not heavily explored until three decades later. Since then, aza-Wittig reaction between iminophosphoranes and aldehydes has become a powerful tool in small molecule organic synthesis strategies due to the absence of metal catalysts, mild reaction conditions, and relatively high yield of the product imine. Small molecule iminophosphoranes can also react with other carbonyl compounds, such as ketones, esters, thioesters, amides, and anhydrides, providing an effective method for construction of C-N bonds, including C=N double bonds (Palacios, Alonso, Aparicio, Rubiales, & de los Santos, 2007). We have previously reported using iminophosphorane intermediates, generated by Staudinger reduction of azides, for synthesis of *O*-acylated 6-amido-(or 6-amino)-6-deoxy-cellulose (Fox & Edgar, 2012; Liu, Liu, Esker, & Edgar, 2016; Marks, Fox, & Edgar, 2016), -curdlan (Zhang & Edgar, 2014), and -pullulan (Pereira & Edgar, 2014) derivatives by *in situ* reaction of the ylide with water, or with excess carboxylic anhydride. The Heinze group has synthesized a class of 6-amino polysaccharide derivatives by an alternate route, using 6-tosylate as the precursor (Genco, Zemljic, Bracic, Stana-Kleinschek, & Heinze, 2012; Heinze, Koschella, Magdaleno-Maiza, & Ulrich, 2001).

Isolated small molecule imines from iminophosphorane-aldehyde condensations can be further reduced by borohydride to produce amines; this is known as indirect reductive amination. In direct reductive amination, imine formation and reduction occur sequentially in one pot, so the imine must be reduced much faster than the carbonyl group of the aldehyde reagent. Therefore the more selective reducing agent $NaCNBH_3$ is preferred for the direct process; in the indirect process its selectivity vs. ester reduction can also be useful (Lane, 1975). There appear to be no previous reports in polysaccharide chemistry of reductive amination between an aldehyde and a polysaccharide iminophosphorane intermediate formed by Staudinger reaction. We hypothesize that the curdlan-based 6-iminophosphorane intermediate generated during the Staudinger reduction may be a sufficiently reactive nucleophile to undergo reductive amination with aldehydes, in the presence of a borohydride reductant, thereby providing a regioselective and chemoselective route to 6-monoalkylamino-6-deoxy-curdlans. Success in this endeavor would complement our recent efforts (Fox & Edgar, 2012; Liu, Liu, Esker & Edgar, 2016; Liu & Edgar, 2017; Marks, Fox & Edgar, 2016; Pereira & Edgar, 2014; Zhang & Edgar, 2014; Zhang, Liu & Edgar, 2016), together affording access to an exceptionally broad array of amines (primary, secondary, tertiary, quaternary ammonium) and amides from either 6-bromo- or 6-azido-6-deoxycurdlan. This synthetic capability would enable flexible structure-property studies with regard to cationic curdlan derivatives, afford useful intermediates for pro-drug synthesis, and feed many other potential applications. Herein we report our attempts to prepare a family of 6-monoalkylamino curdlans by reacting 6-azido-6-deoxy-2,4-di-*O*-acyl-curdlan with Ph_3P and aldehydes (**Scheme 1**).

2. Experimental

2.1 Materials

Curdlan (DP ~ 500) was obtained from Wako Chemicals and dried under vacuum at 40 °C overnight prior to use. Lithium bromide (LiBr, laboratory grade, Fisher) was dried under vacuum at 125 °C. *N*-Bromosuccinimide (NBS, 99%, Acros) was recrystallized from boiling water and dried for two days under reduced pressure over anhydrous calcium chloride. *N,N*-Dimethylacetamide (DMAc, reagent grade, Fisher) and *N*,*N*-dimethylformamide (DMF, HPLC grade, Fisher) were stored over 4 Å molecular sieves. Tetrahydrofuran (THF, 99.8%, extra dry, stabilized, AcroSeal®), *N*-methyl-2-pyrrolidone (NMP, 99.5%, extra dry, AcroSeal®), pyridine (Pyr, anhydrous, 99%, AcroSeal®), benzaldehyde (PhCHO, purified by redistillation, ≥ 99.5%, Aldrich), 4-nitrobenzaldehyde (4-NO_2PhCHO, 98%, Aldrich), 4-chlorobenzaldehyde (4-ClPhCHO, 98%, Aldrich), 2-pyridinecarboxaldehyde (Pyr-2-CHO, 99%, Aldrich), 4-dimethylaminopyridine (DMAP, Acros), triphenylphosphine (Ph_3P, 99%, Acros), sodium azide (NaN_3, 99%, Alfa Aesar), sodium hydroxide (NaOH, reagent grade, 97%, Sigma-Aldrich), sodium cyanoborohydride ($NaBH_3CN$, reagent grade, 95%, Aldrich), acetic anhydride (Ac_2O, 99+%, Sigma-Aldrich), propionic anhydride (Pr_2O, 97% Sigma-Aldrich), *n*-butyric anhydride (Bu_2O, 98%, Acros), ethanol (HPLC grade, Fisher), molecular sieves (4 Å, Fisher) and regenerated cellulose dialysis tubing (MW 3500, Fisher) were used as received.

2.2 Measurements

^1H, ^{13}C and HSQC NMR spectra were obtained on a Bruker Avance II 500MHz spectrometer in $CDCl_3$, DMSO-d_6, DMF-d_7, or D_2O at room temperature or 50 °C, employing 32, 15,000 and 19,000 scans, respectively. Infrared spectroscopic analyses of samples as pressed KBr pellets were obtained

on a Thermo Electron Nicolet 8700 instrument using 64 scans and 4 cm^{-1} resolution. Carbon and nitrogen contents were determined by Micro Analysis Inc. using a Perkin Elmer 2400 II analyzer.

2.3 Methods

2.3.1 Synthesis of 6-bromo-6-deoxycurdlan in DMAc/LiBr

The procedure was adapted from a previously reported method (Zhang & Edgar, 2014). Dried curdlan (4.00 g, 24.7 mmol AGU) was dissolved in DMAc (110 mL) and LiBr (36.00 g, 42.4 mmol). Separate solutions of Ph_3P (25.96 g, 4 eq per AGU) and NBS (17.58 g, 4 eq per AGU), each in dry DMAc (50 mL), were added dropwise, sequentially, to the curdlan solution. The reaction solution was then heated at 70 °C for 1 h. The mixture was added slowly to 1 L of a 50:50 mixture of methanol and deionized water and then filtered to recover the precipitate. The isolated sample was washed with ethanol twice and then dried under vacuum (40 °C) overnight to yield 6-bromo-6-deoxycurdlan. ^{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). Yield: 86%.

2.3.2 Synthesis of 6-azido-6-deoxycurdlan

The procedure was adapted from one reported earlier (Zhang & Edgar, 2014). Briefly, dry 6-bromo-6-deoxycurdlan (1.00 g, 4.44 mmol) was dissolved in DMSO (25 mL). Then NaN_3 (1.44 g, 5 eq per AGU) was added to the solution. The resulting mixture was heated at 80 °C for 24 h under nitrogen. The product was isolated by pouring into 300 mL of deionized water and collected by filtration. The precipitate was re-dissolved in acetone, re-precipitated into deionized water, and again isolated by filtration. The sample was dried under vacuum (40 °C) overnight to yield 6-azido-6-deoxycurdlan. ^{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). Yield: 92%.

2.3.3 Synthesis of 6-azido-6-deoxy-2,4-di-*O*-acyl-curdlan

The procedure was adapted from one reported earlier (Zhang & Edgar, 2014). Dry 6-azido-6-deoxycurdlan (1.00 g, 5.35 mmol), 4-dimethylaminopyridine (DMAP, 20 mg), pyridine (3.6 mL, 10 eq per AGU), and 20 eq per AGU of carboxylic anhydride (Ac_2O, 10.1 mL; Pr_2O, 13.8 mL) were combined. The mixture was stirred at 80 °C for 24 h, then cooled and added slowly to 200 mL deionized water to precipitate the product, which was recovered by filtration, re-dissolved in chloroform, re-precipitated into ethanol, and finally isolated by filtration. The product was washed with ethanol and water several times and then dried under vacuum (40 °C) overnight.

6-Azido-6-deoxy-2,4-di-*O*-acetyl-curdlan: ^{1}H NMR ($CDCl_3$) **(Fig. S2a)**: δ 4.7 (H-5), 4.6 (H-5), 4.3 (H-1), 3.7 (H-6), 3.5 (H-6'), 3.3 (H-3), 3.1 (H-2), 2.2-1.9 (CH_3-acetate). Yield: 91%.

6-Azido-6-deoxy-2,4-di-*O*-propionyl-curdlan: ^{1}H NMR ($CDCl_3$) (**Fig. S2b**): δ 4.7 (H-5), 4.6 (H-5), 4.3 (H-1), 3.7 (H-6), 3.5 (H-6'), 3.3 (H-3), 3.1 (H-2), 2.6-2.0 (CH_2-propionate), 1.3-1.0 (CH_3-propionate). Yield: 90%.

2.3.4 Syntheses of (6-amino-*N*-benzylidene/4-nitrobenzylidene/4-chlorobenzylidene/2-pyridinylmethylene)-6-deoxy-2,4-di-*O*-acetyl-curdlans

Dry 6-azido-6-deoxy-2,4-di-*O*-acetyl-curdlan (0.25 g, 0.92 mmol) was dissolved in 15 mL of THF or DMAc in a 50 mL flask with molecular sieves. Then Ph_3P (2 eq per AGU) and 30 eq per AGU of aldehyde (PhCHO (2.94 g), 4-NO_2PhCHO (4.17 g), 4-ClPhCHO (3.88 g), or Pyr-2-CHO (2.96 g)) were added to the flask. The solution was stirred under nitrogen at room temperature for 24 h. The solution was transferred to 3,500 g/mol molecular weight cutoff (MWCO) dialysis tubing that was

then placed in a large beaker containing ethanol. After three to five days of dialysis, the precipitate formed within the tubing was isolated by filtration and then dried under vacuum (40 °C) overnight. The DS_{imine} values were determined according to **Eq. (1)** by ^{1}H NMR.

$$DS_{imine} = \frac{7 \times I_{H,aromatic+H,7}}{6 \times I_{H,AGU}} \quad \textbf{(1)}$$

I = integral, $H_{,aromatic}$ = aromatic protons, $H_{,AGU}$ = curdlan backbone protons

6-Amino-*N*-benzylidene-6-deoxy-2,4-di-*O*-acetyl-curdlan: ^{1}H NMR ($CDCl_3$): δ 8.2 (H-7), 7.7 (H-9, 13), 7.5 (H-10~12), 5.2-3.2 (curdlan backbone protons H-1~6), 2.2-1.8 (CH_3-acetate); ^{13}C NMR ($CDCl_3$): δ 169 (C=O-acetate), 162 (N=C-7), 145-125 (aromatic carbons C-8~13), 100 (C-1), 82-68 (C-2~5), 62 (C-6-N), 20 (CH_3-acetate). Yield: 90%. Elemental analysis: %C 57.25, %H 5.38, %N 4.40 (theoretical (DS 1.0) %C 61.26, %H 5.71, %N 4.20); $DS_{imine, EA}$ = 0.93.

6-Amino-*N*-4-nitrobenzylidene-6-deoxy-2,4-di-*O*-acetyl-curdlan: ^{1}H NMR ($CDCl_3$): δ 8.3 (H-10, 12), 7.9 (H-7, 9, 13), 4.9-3.4 (curdlan backbone protons H-1~6), 2.2-1.8 (CH_3-acetate). ^{13}C NMR (DMSO-d_6): δ 170 (C=O-acetate), 162 (C-7=N), 150-120 (aromatic protons C-8~13), 100 (C-1), 82-68 (C-2~5), 60 (C-6-N), 21 (CH_3-acetate). Yield: 81%.

6-Amino-*N*-4-chlorobenzylidene-6-deoxy-2,4-di-*O*-acetyl-curdlan: ^{1}H NMR ($CDCl_3$): δ 8.1 (H-7), 7.6 (H-9, 13), 7.4 (H-10, 12), 5.0-3.3 (curdlan backbone protons H-1~6), 2.3-1.8 (CH_3-acetate). ^{13}C NMR ($CDCl_3$): δ 21 (CH_3-acetate), 61 (C-6-N), 70-105 (curdlan backbone carbons), 125-145 (aromatic carbons), 162 (C-7=N), 169 (C=O-acetate). Yield: 87%.

6-Amino-*N*-2-pyridinylmethylene-6-deoxy-2,4-di-*O*-acetyl-curdlan: ^{1}H NMR ($CDCl_3$): δ 7.5 (H-10, 11, 12), 7.7 (H-9, 13), 8.2 (H-7), 5.2-3.2 (curdlan backbone protons H-1~6), 2.2-1.8 (CH_3-acetate). ^{13}C NMR ($CDCl_3$): δ 21 (CH_3-acetate), 61(C-6-N), 70-105 (curdlan backbone carbons), 120-160 (aromatic carbons), 164 (C-7=N), 169 (C=O-acetate). Yield: 84%.

2.3.5 Synthesis of 6-amino-*N*-benzyl-6-deoxy-2,4-di-*O*-acetyl-curdlan

Dry 6-amino-*N*-benzylidene-6-deoxy-2,4-di-*O*-acetyl-curdlan (0.1 g, 0.30 mmol) was dissolved in 10 mL of THF in a 50 mL flask. Then $NaBH_3CN$ (0.19 g, 3.0 mmol, 5 eq per AGU) was added to the flask. The solution was stirred at ambient temperature (ca. 23°C) for 24 h. The mixture was added to 100 mL of ethanol. The precipitate was isolated by filtration and washed with ethanol, then dried under vacuum (40 °C) overnight. The DS_{amine} values were determined according to **Eq. (2)** by ^{1}H NMR. Elemental analysis: %C 56.46, %H 5.45, %N 4.78 (theoretical (DS 1.0) %C 60.89, %H 6.27, %N 4.18); $DS_{imine,\ EA}$=0.35.

$$DS_{amine} = 1 - \frac{5 \times I_{H,7}}{I_{H,aromatic}} \qquad \textbf{(2)}$$

6-Amino-*N*-benzyl-6-deoxy-2,4-di-*O*-acetyl-curdlan: ^{1}H NMR ($CDCl_3$): δ 8.2 (H-7), 7.7 (H-9, 9', 13, 13'), 7.5 (H-10~12. 10'~12'), 4.8-3.2 (curdlan backbone protons H-1~6 & H-7'), 2.2-1.8 (CH_3-acetate).

2.3.6 Synthesis of 6-amino-*N*-benzyl-6-deoxy-2,4-di-*O*-acetyl-curdlan by one-pot reductive amination via Staudinger ylide

Dry 6-azido-6-deoxy-2,4-di-*O*-acetyl-curdlan (0.25 g, 0.92 mmol) was dissolved in 15 mL of DMF in a 50 mL flask containing molecular sieves (2 g, 4 Å). Then Ph_3P (2 eq per AGU), PhCHO (2.82 mL,

30 eq per AGU), and $NaBH_3CN$ (0.23 g, 10 eq per AGU) were added to the flask. The solution was stirred under nitrogen at ambient temperature for 24 h. The solution was transferred to 3,500 g/mol MWCO dialysis tubing and dialyzed against ethanol for at least three days; the ethanol was replaced daily. The precipitate formed within the tubing was isolated by filtration and then dried under vacuum (40 °C) overnight. The DS_{amine} values were determined according to **Eq. (3)** by ^1H NMR.

$$DS_{amine} = (\frac{5 \times I_{H,AGU+H,7}}{I_{H,aromatic}} - 7)/2 \quad \textbf{(3)}$$

6-Amino-*N*-benzyl-6-deoxy-2,4-di-*O*-acetyl-curdlan: ^1H NMR ($CDCl_3$): δ 7.8-7.2 (aromatic protons H-9~13), 5.1-2.8 (curdlan backbone protons H-1~6 & H-7). Yield: 69%.

2.3.7 Synthesis of 6-amino-*N*-benzyl-6-deoxycurdlan by one-pot reductive amination via Staudinger ylide

Dry 6-azido-6-deoxycurdlan (0.25 g, 1.34 mmol) was dissolved in 15 mL of THF in a 50 mL flask containing molecular sieves (2 g, 4 Å). Then Ph_3P (2 eq per AGU), PhCHO (2.82 mL, 30 eq per AGU), and $NaBH_3CN$ (3.36 g, 40 eq per AGU) were added to the flask. The clear solution was stirred under nitrogen at ambient temperature for 24 h. The solution was transferred to 3,500 g/mol MWCO dialysis tubing and dialyzed against ethanol for at least three days; the ethanol was replaced daily. The precipitate formed within the tubing was isolated by filtration and then dried under vacuum (40 °C) overnight. The DS_{amine} values were determined according to **Eq. (3)** by ^1H NMR.

6-Amino-*N*-benzyl-6-deoxycurdlan: ^1H NMR ($CDCl_3$): δ 7.6-7.3 (aromatic protons H-9~13), 5.1-2.8 (curdlan backbone protons H-1~6 & H-7). Yield: 75%.

3 Results and discussion

3.1 Synthesis of 6-amino-*N*-benzylidene-6-deoxy-2,4-di-*O*-acyl-curdlan

The Furuhata bromination and iminophosphorane ylide-based approaches previously reported by our laboratory provided ready access to many 6-amino-, 6-ammonio-, and 6-amido-6-deoxy curdlan derivatives, but could not provide access to secondary amines. Secondary amines can be challenging to synthesize, since alkylation of a primary amine precursor frequently affords a mixture of the corresponding secondary and tertiary amines, and quaternary ammonium, due to insufficiently differentiated starting material and product reactivity (Salvatore, Yoon & Jung, 2001). We felt that a reductive amination protocol, previously unreported from polysaccharide-linked Staudinger ylides, could be an effective approach to these secondary amines. Herein, we prepared a family of 6-monoalkylamino curdlan derivatives by reacting 6-azido-6-deoxy-2,4-di-*O*-acyl-curdlan with Ph_3P and aldehydes (**Scheme 1**). In the event, we found that treating 2,4-*O*-acetyl-6-azido-6-deoxycurdlan in THF with triphenylphosphine at ambient temperature to form the iminophosphorane ylide as we had before, but this time in the presence of benzaldehyde as electrophile, afforded 6-amino-*N*-benzylidene-6-deoxy-2,4-di-*O*-acetyl-curdlan; product identity was confirmed by FTIR and $^{1}H/^{13}C$ NMR spectroscopy as described in detail below. FTIR analysis (**Fig. S10**) showed a characteristic imino C=N stretch at 1671 cm^{-1} as well as aromatic C-H bend at 762 cm^{-1} and 701 cm^{-1}, indicating successful amino-*N*-benzylidene introduction. No azido N_3 stretch was observed around 2100 cm^{-1}. Conversion to the imine appeared to be high but incomplete under these conditions, as indicated by ^{1}H NMR integration, so we sought to optimize the reaction conditions to enhance conversion. We explored the effects of changing solvent (DMAc *vs.* THF), temperature (RT *vs.* 50 °C), reaction time (24 h *vs.* 36 h) and benzaldehyde molar excess (20 *vs.* 30 equiv. per AGU). As can be seen from **Table S1 entries 1** and **2**, reaction in THF gave slightly higher DS_{imine} than in DMAc. Extending the reaction time by 12 h did not enhance conversion as might have been expected; instead, DS_{imine}

decreased from 0.89 (**entry 1**) to 0.73 (**entry 3**). We feel that the most logical explanation is hydrolysis of the initially formed imine due to the presence of adventitious water. Note that, even when azide reduction is complete as it appears to be here, there are two potential sources of an unsubstituted 6-amino-6-deoxy byproduct (that is, 6-amino-6-deoxy-2,4-di-*O*-acetyl-curdlan). This product can arise by hydrolysis of the imine product (from co-product water, or adventitious water), or alternatively by protonation of the intermediate iminophosphorane ylide by water (as in the intentional synthesis of the primary amine by running the Staudinger reduction in water as solvent or co-solvent (Fox & Edgar, 2012; Pereira & Edgar, 2014; Zhang & Edgar, 2014)). Increasing either the excess of PhCHO (**entry 4**) or the reaction temperature (**entry 5**) afforded slightly increased DS (DS_{imine} 0.91), approaching full conversion (DS_{Br} of the starting 6-bromo product was 0.95). Due to the potential for degradation and instability of imino derivatives at higher temperatures, we applied the reaction conditions of **entry 4** for the following imine syntheses.

^{13}C NMR was useful for characterizing the 6-amino-*N*-benzylidene-6-deoxy-2,4-di-*O*-acetyl-curdlan product (**Fig. 1**); resonances in the range of δ 126-137 ppm were assigned to the aromatic carbons of the phenyl ring. Additionally, signals at δ 62 and 162 ppm were assigned to the **C**$_6$-N and N=**C**$_7$ respectively. Diagnostic features of the ^1H NMR spectrum (**Fig. S3**) included the aromatic proton resonances at δ 7.5 ppm ($H_{10,11,12}$) and δ 7.7 ppm ($H_{9,13}$) as well as the imino proton signal at δ 8.2 ppm (H_7) from the **H**-C_7=N moiety. DS_{imine} values were calculated by the ratio of the intregral of protons $H_{7\text{-}13}$ to that of curdlan backbone protons ($H_{1\text{-}6/6'}$) and are summarized in **Table 1**. Our previous work indicated that the Staudinger reaction (Ph_3P) to reduce the curdlan azide to amine is sufficiently mild to preserve ester bonds against reduction (Zhang & Edgar, 2014); we confirmed that they were also stable during imine formation by the presence in the product ^1H NMR spectrum of singlets from of acetyl groups around δ 2.0 ppm, integration of which indicated full 2, 4-*O*-

substitution (DS_{Ac} 2.0). However, there were some small peaks evident close to the imino aromatic signals as well as the apparent aldehyde proton signal at δ 10.0 ppm and the carbonyl carbon signal at δ 192.0 ppm, attributed to a residual benzaldehyde impurity; this is likely to be the result of imide hydrolysis rather than or in addition to failure to separate excess benzaldehyde from the product by dialysis. In order to increase the DS and avoid the accompanying hydrolysis by co-product or adventitious water, we hypothesized that inclusion of molecular sieves in the reaction mixture would capture any water present, thereby minimizing imine hydrolysis. When molecular sieves were so used, benzaldehyde signals were absent in the products, as evidenced by the ^{1}H NMR spectra (**Fig. 2**) with complete imine substitution (DS_{imine} 1.0), confirming our hypothesis and providing a highly efficient route to the regioselectively substituted imines.

In order to explore the breadth of applicability of this 6-deoxy-6-iminocurdlan synthetic method, three other aldehydes (4-NO_2PhCHO, 4-ClPhCHO and 2-PyrCHO) were reacted with 6-azido-6-deoxycurdlan under otherwise identical reaction conditions (**Scheme 1**). ^{13}C NMR spectroscopy (**Fig. S7-9**) helped to confirm product structures. Resonances in the range of δ 120-160 ppm were assigned to the aromatic carbons, while peaks around 60 ppm (imino-substituted C6) and 162 ppm (imine carbon, C7) confirmed successful curdlan aryl imine formation. The imines were also characterized by ^{1}H NMR spectroscopy (**Fig. S4**). In each case, curdlan backbone proton resonances fell within δ 5.0-3.0 ppm while aromatic ring and **H**-C=N-R protons resonated in the range of δ 8.7-7.4 ppm. ^{1}H and ^{13}C NMR spectra indicated that the isolated imine products were free of azide or brominated impurities within the sensitivities of the techniques. **Table 1** summarizes the chemical structure, DS_{imine}, product yield, and chemical shift assignments of the imino analogs. Degrees of substitution of the imino products were determined as $DS_{ClPh\text{-}imine}$ 0.63, $DS_{Pyr\text{-}imine}$ 0.76 and

$DS_{NO2Ph\text{-}imine}$ 0.77, respectively. The apparent incomplete imine formation could rather be due to partial hydrolysis of the initially formed imine (each derivative has a more electron poor, thus more reactive imine moiety than that of the *N*-benzylidene-6-amino derivative).

3.2 Borohydride reduction of 6-amino-*N*-benzylidene-6-deoxy-2,4-di-*O*-acetyl-curdlan

We selected sodium cyanoborohydride for initial experiments on reduction of the imine to secondary amine, since it is known to be less reactive towards ester groups than $NaBH_4$ (Boechat, da Costa, de Souza Mendonça, de Oliveira, & Vinícius Nora De Souza, 2004; Borch, Bernstein, & Durst, 1971; Das, Roy, & Das, 2004; Lane, 1975). Therefore we treated 2,4-di-*O*-acetyl-(6-amino-*N*-benzylidene)-6-deoxycurdlan (DS_{imine} 1.0) with different molar ratios of $NaBH_3CN$ to attempt imine to amine reduction. As clearly indicated by ^1H NMR spectra and integration (**Fig. S5)**, the amine product was obtained but with DS_{amine} only 0.18 (**Table 2**, **entry 1)**, far lower than the starting DS_{imine} 1.0. We could increase amine DS to 0.38 (**Table 2**, **entry 2**) by increasing reaction time from 5 h to 24 h, indicating that incomplete reduction was at least part of the problem. On the other hand, increasing the $NaBH_3CN$ molar excess had essentially no effect on DS_{amine}, which leveled off at around 0.4 (**Table 2**, **entries 3-5**).

3.3 One-pot reductive amination via Staudinger ylide

We initially explored the two-step process for conversion of iminophosphorane to secondary amine (imine formation, followed by separate reduction) in order to make sure we understood each step and the influence of reaction conditions on each. We hoped however that ultimately a one-pot process of imine formation and reduction would be successful, as is commonly employed in small molecule chemistry (Abdel-Magid, Carson, Harris, Maryanoff, & Shah, 1996; Goldstein & Cross, 2015; Sato, Sakamoto, Miyazawa, & Kikugawa, 2004). We hypothesized that a one-pot method, by

avoiding isolation of the hydrolytically sensitive imine, would provide improved overall selectivity and efficiency. Our one-pot reductive amination started by generating a Staudinger iminophosphorane from the corresponding azide in the presence of molecular sieves, then reacting with excess aldehyde in the presence of sodium cyanoborohydride, by a method in which all reagents (2,4-*O*-acetyl-6-azido-6-deoxycurdlan, Ph_3P, PhCHO, $NaBH_3CN$) were present from the beginning in various solvent systems. We investigated the influence of solvent upon the reduction; reduction proceeded in common organic solvents including THF, NMP and DMF (**Table 3, entry 1-3**). The DS_{amine} value reached 0.5 when the reduction was carried out in DMF, while DS 0.27 and 0.31 were achieved in THF and NMP, respectively. We also noted that DS_{amine} obtained was much higher (0.89) when 6-azido-6-deoxycurdlan, lacking the ester moieties at O-2 and O-4, was used as starting material (**Table 3, entry 4**). 6-Azido-6-deoxycurdlan has better solubility than its acetylated analog in THF solvent, which may influence reduction conversion. The electron withdrawing ester moieties may also influence the rate of ylide attack upon the aldehyde, and the stability of the intermediate ylide to hydrolysis, both of which could negatively influence DS_{amine}. **Fig. 3** clearly shows the broad aromatic protons in the range of δ 7.5 - 7.3 ppm with disappearance of the imino proton at δ 8.2 ppm, indicating high conversion to *N*-benzylidene-6-amino-6-deoxycurdlan. We note that the spectrum also shows a small amount of residual PPh_3 and/or triphenyl phosphine oxide (7.6 – 8.0 ppm). It is well understood that it can be difficult to remove such residues, especially from polymeric products; our previous work has demonstrated that they are typically removed in subsequent steps (Fox & Edgar, 2012; Zhang & Edgar, 2014). In addition, the FTIR spectrum (**Fig. S11**) demonstrated a significant absorption at 3200 - 3600 cm^{-1}, assigned to N-H stretch of the amine product. This confirmed our hypothesis that hydrolytic instability of the imine had been the limiting factor in the two-pot, imine isolation approach, and provided a far more

efficient route from 6-azido-6-deoxycurdlan to the corresponding 6-(benzylamino)-6-deoxycurdlan derivative.

4. Conclusions

We have developed methods for further exploitation of the nucleophilic Staudinger ylide (iminophosphorane intermediate) obtained by reduction of 6-azido-6-deoxycurdlans for the synthesis of 6-aminated curdlans, specifically by reductive amination. By adding aldehydes and/or sodium cyanoborohydride, a series of imino- and amino-curdlans was produced with high chemoselectivity, providing a new strategy for regioselective incorporation of a range of monoalkylamino pendants at C-6 of curdlan.

The success and selectivity of these approaches complement synthetic strategies previously developed in our lab for preparing families of regioselectively aminated curdlan derivatives, now providing access to a very broad variety of amines (primary, secondary, and tertiary amines, and quaternary ammonio derivatives) and amides (with amide acyl moieties the same as those appended to the OH groups as esters, different from those moieties, or with only amides and no esters present, whichever is preferred). All of these amino, ammonio, and amido curdlans are prepared from 6-bromo- or 6-azido-6-deoxycurdlan (**Scheme 2**) with very high degrees of chemo- and regioselectivity. This set of complementary synthetic methods opens doors to a wide variety of potentially useful aminated/amidated polymers for use biomedical, pharmaceutical, and other fields, and should of course be applicable to other polysaccharides, for example other glucans with unencumbered C-6 OH groups. We will continue to explore new ways to exploit the nucleophilic Staudinger ylide for regio- and chemoselective synthesis of substituted, functional, and useful polysaccharide derivatives.

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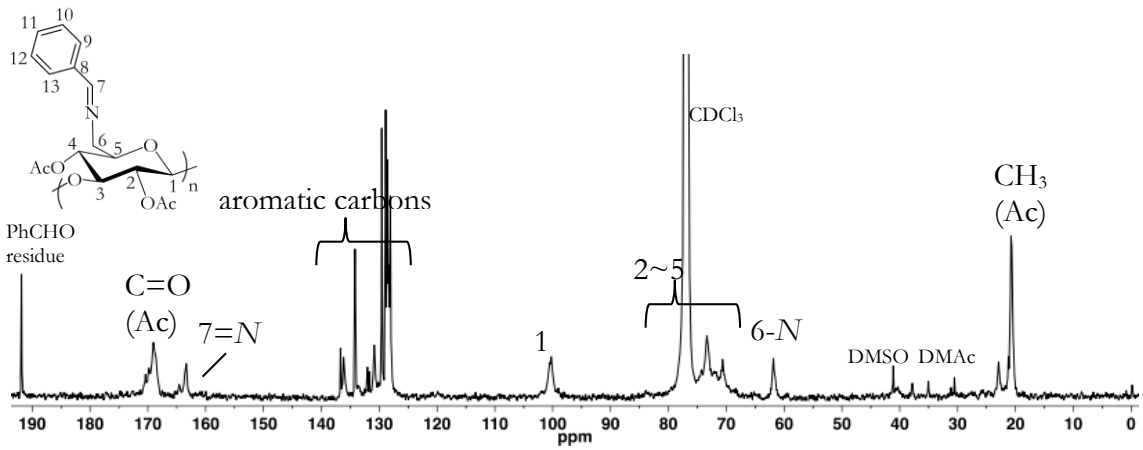


Fig. 1. ^{13}C NMR spectrum of 6-amino-*N*-benzylidene-6-deoxy-2,4-di-*O*-acetyl-curdlan performed in $CDCl_3$ at RT (30 equiv. PhCHO/AGU, THF, RT, 24 h, DS_{imine} = 0.91).

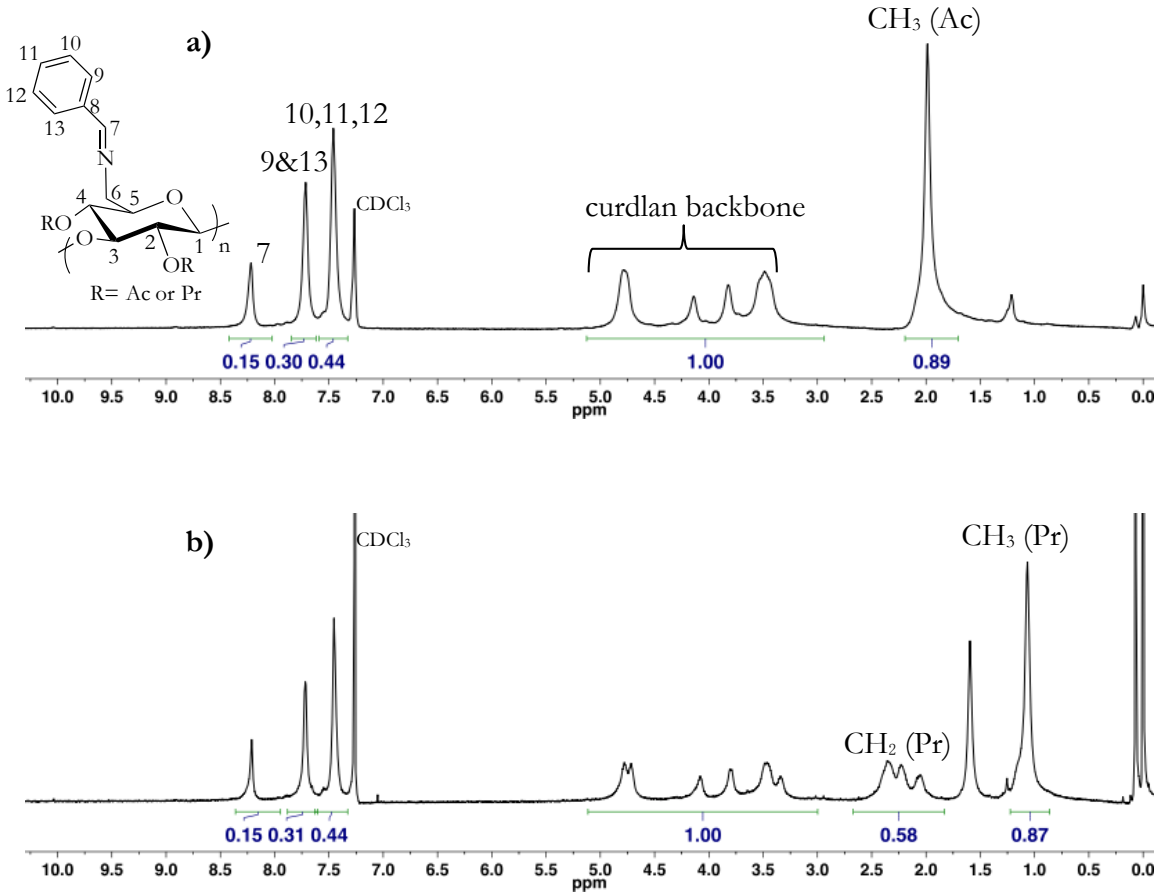


Fig. 2. ^{1}H NMR spectra (performed in $CDCl_3$ at RT) of **(a)** 6-amino-*N*-benzylidene-6-deoxy-2,4-di-*O*-acetyl-curdlan and **(b)** 6-amino-*N*-benzylidene-6-deoxy-2,4-di-*O*-propionyl-curdlan (30 equiv. PhCHO/AGU, THF, RT, 24 h, molecular sieves, DS_{imine} = 1.0 (conversion 100%)).

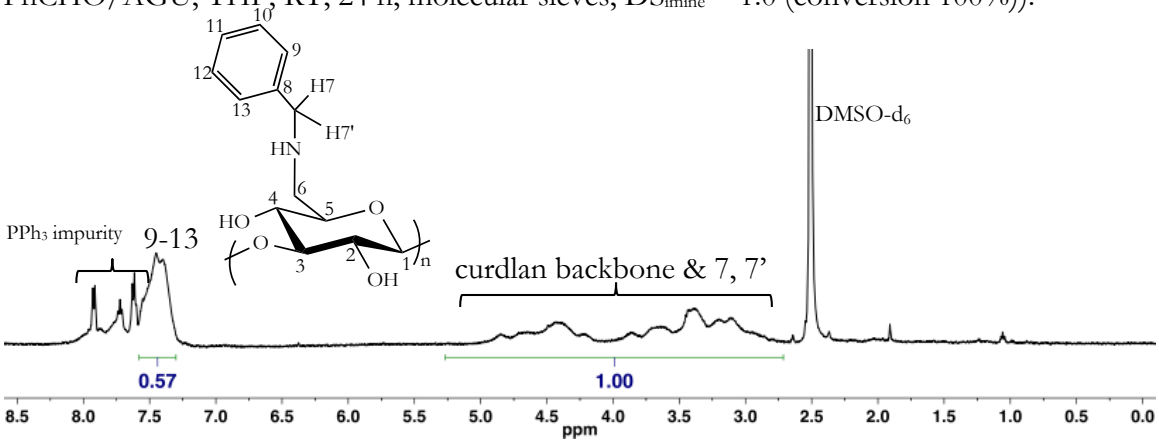
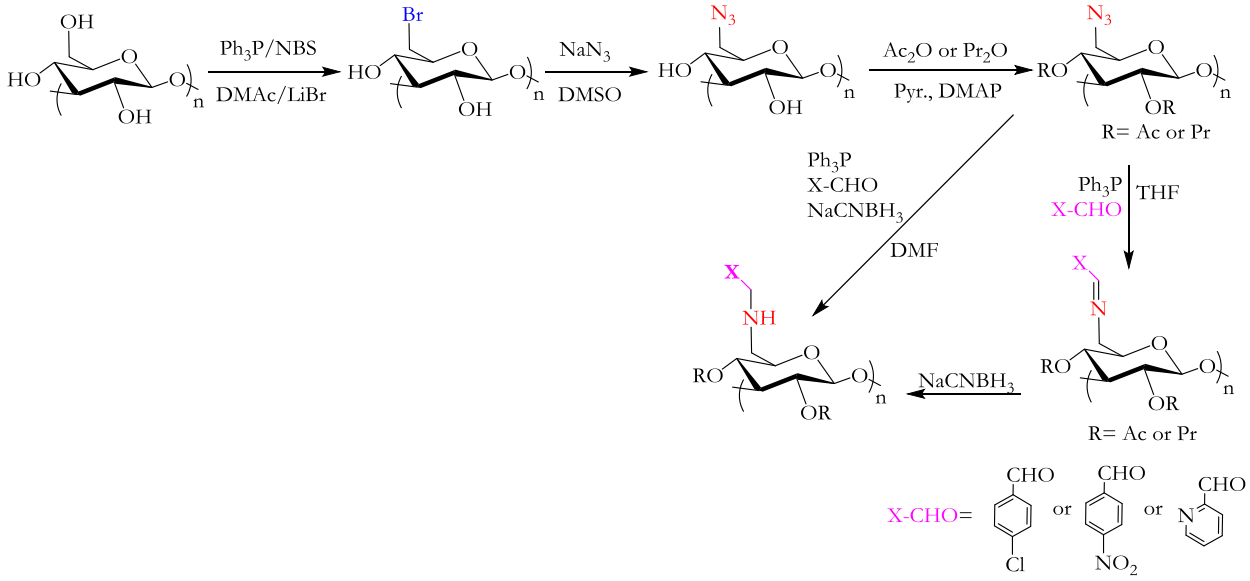
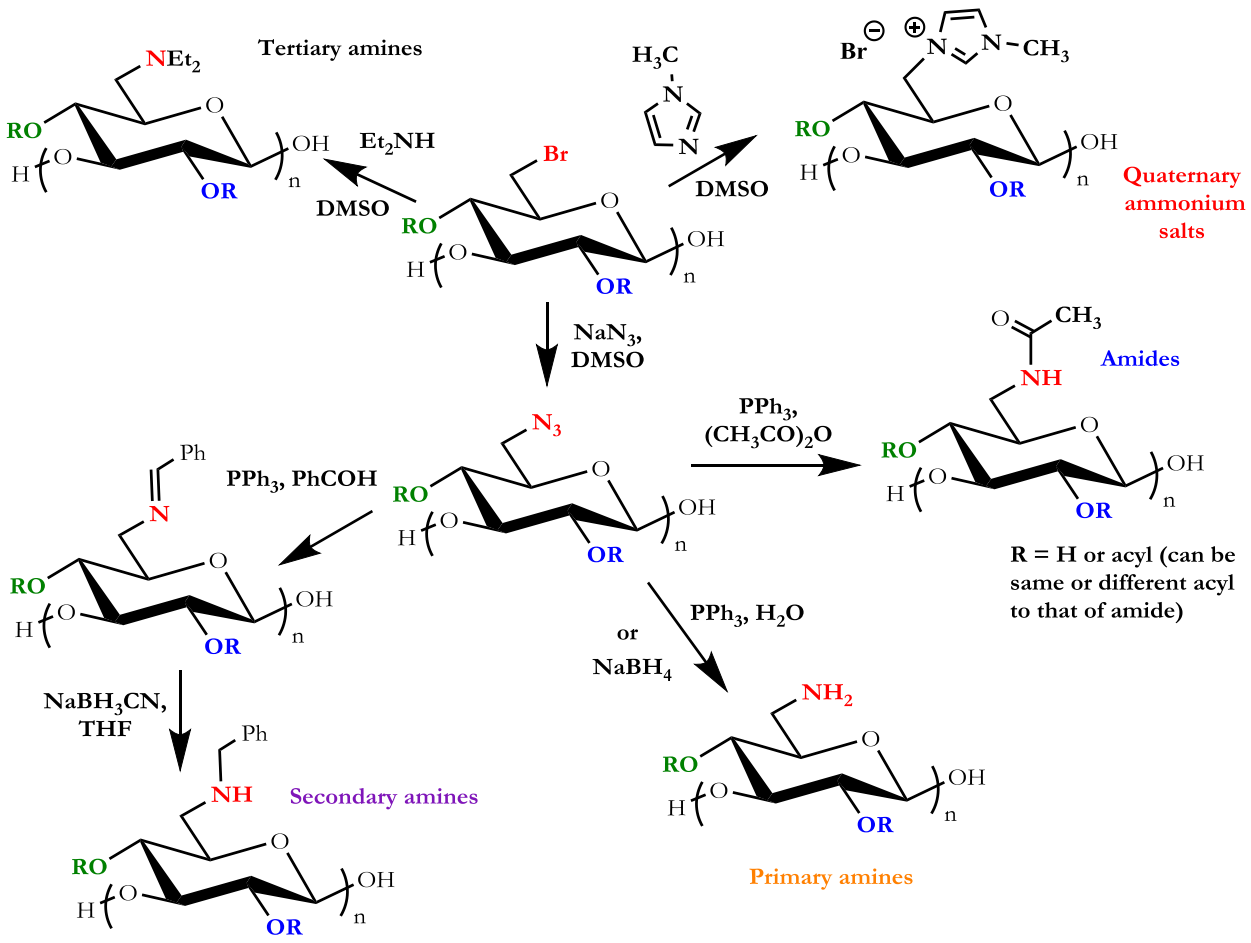


Fig. 3. ^{1}H NMR spectrum (performed in DMSO-d_6 at RT) of 6-amino-*N*-benzyl-6-deoxycurdlan (**Table S1, entry 4**).



Scheme 1. Synthetic scheme for 6-aminated curdlan derivatives via Staudinger ylide.



Scheme 2. Example syntheses of curdlan derivatives regioselectively aminated/amidated at C-6.

Table 1. ^{1}H NMR chemical shift assignments for aromatic ring and imine protons, DS_{imine} values, and yields of 6-deoxy-6-imino-curdlans.

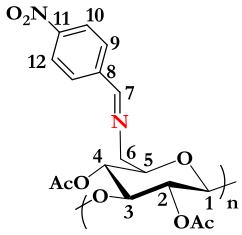
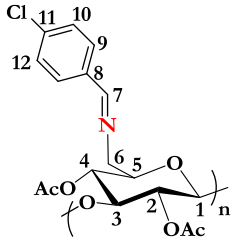
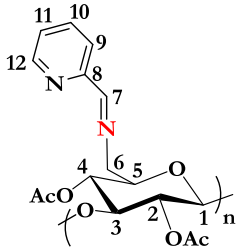
Imine structure	**Aldehyde used**	**^{1}H NMR assignment**		**DS_{imine}**	**Yield (%)**
		proton	**δ (ppm)**		
	4-O_2NPhCHO	10. 12	8.3	0.77	81
		7. 9. 13	7.9		
	4-ClPhCHO	7	8.1	0.63	87
		9. 13	7.6		
		10. 12	7.4		
	Pyr-2-CHO	12	8.6	0.76	84
		7. 9	8.3-8.2		
		10. 11	7.7-7.9		

Table 2. Substitution achieved (DS_{amine}) *vs.* $NaBH_3CN$ imine reduction conditions.*

Entry	$NaBH_3CN$ (eq/AGU)	Solvent	Temp. (°C)	Time (h)	DS_{amine}
1	2	THF	RT	5	0.15
2				24	0.38
3	5				0.41
4	10				0.38
5	20				0.42

*Isolated 6-amino-*N*-benzylidene-6-deoxy 2,4-di-*O*-acetyl-curdlan (DS_{imine} 1.0) used as starting material.

Table 3.

Substitution achieved (DS_{amine}) by one-pot reductive amination of the Staudinger ylide.*

Entry	$NaBH_3CN$ (eq/AGU)	Solvent	Temp. (°C)	Time (h)	DS_{amine}
1	10	THF	RT	24	0.27
2		NMP			0.31
3		DMF			0.50
4	40	THF			0.89

*2,4-Di-*O*-acetyl-6-azido-6-deoxycurdlan used as starting material (**entries 1-3**) except **entry 4** (used 6-azido-6-deoxycurdlan), with Ph_3P (2 eq/AGU), PhCHO (30 eq/AGU).