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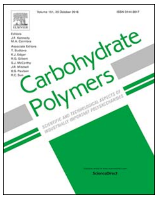

Selective synthesis of curdlan ω-carboxyamides by Staudinger ylide nucleophilic ring-opening

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ABSTRACT

Chemoselective modification of polysaccharides is a significant challenge, and regioselective modification is even more difficult, due to the low and similar reactivity of the various polysaccharide hydroxyl groups. Bromination of glycans that possess free 6-OH groups is exceptional in that regard, giving regiospecific, high-yield access to 6-bromo-6-deoxyglycans. Herein we report a simple and efficient pathway for synthesizing 6-ω-carboxyalkanamido-6-deoxy-containing polysaccharide derivatives in a sequence starting from 6-bromo-6-deoxycurdlan, via azide displacement, then conversion of the azide to the iminophosphorane ylide by triphenylphosphine (Ph_3P). We take advantage of the nucleophilicity of the iminophosphorane nitrogen by subsequent regioselective ring-opening reactions of cyclic anhydrides. These reactions of the useful polysaccharide curdlan were essentially completely regio- and chemo-selective, proceeding under mild conditions in the presence of ester groups, yet preserving those groups. These interesting polysaccharide-based materials have pendant carboxyls attached through a hydrocarbon tether and hydrolytically stable amide linkage; as such they are promising for diverse application areas, including aqueous dispersions for coatings, adhesives, and other consumer products, and for amorphous solid dispersions in oral drug delivery.

1. Introduction

Carboxyl-containing polysaccharides are useful in many applications, being based on abundant, renewable, often biodegradable, and diverse polysaccharides. Pendent carboxyl groups impart pH responsiveness, and can enhance specific interactions with small molecules, enable dissolution or dispersion in water, and stabilize the polysaccharide itself or other molecules with which it associates against aggregation, due to the polyelectrolytic nature of the carboxylated polysaccharide. Despite their utility, there are not many effective methods for making carboxylated polysaccharides. Existing methods include direct oxidation of polysaccharides that contain unsubstituted C-6 primary hydroxyls (de Nooy, Besemer, van Bekkum, van Dijk, & Smit, 1996; Tamura, Wada, & Isogai, 2009; Pereira, Mahoney, & Edgar, 2014), reaction with chloroacetic acid to form carboxymethyl derivatives (Heinze, Koschella, Brackhagen, Engelhardt, & Nachtkamp, 2006; Posey-Dowty et al., 2007), cross-metathesis to introduce carboxy-terminal substituents (Meng, Matson, & Edgar, 2014), and ring opening of cyclic anhydrides by polysaccharide hydroxyls (Hollabaugh, Burt, & Walsh, 1945; Liu et al., 2007; McCormick & Dawsey, 1990; Heinze & Koschella, 2005; Liu, Kar, & Edgar, 2012). Each has limitations; C-6 oxidation gives no flexibility with regard to length of the tether to the polysaccharide chain, and is limited to a maximum DS of 1.0. Carboxymethylation is very useful; carboxymethylcellulose (CMC) has the largest cellulose ether market, including in detergent applications (Benchabane & Bekkour, 2008). However it is quite difficult to achieve high DS(CM) (> ca. 1.5) (Baar, Kulicke, Szablikowski, & Kiesewetter, 1994), due to developing repulsion between appended carboxymethyl groups and approaching chloroacetate electrophiles, both of which are anionic under standard CMC reaction conditions. Ring opening of cyclic anhydrides by *O*-centered nucleophiles is more flexible (though limited by stability of the needed anhydrides, with adipic being the practical maximum ring size), but affords hydrolytically labile ester linkages (Malm & Fordyce, 1940). Thus cellulose succinates have been prepared by ring-opening of succinic anhydride by cellulose or cellulose esters with or without an organic base catalyst (Hollabaugh et al., 1945; Edgar, 1993), while the commercial enteric polymer cellulose acetate phthalate has been produced by base-catalyzed reaction of cellulose acetate with phthalic anhydride (Malm & Fordyce, 1940). These hydrolytically labile ester linkages might be adequate to survive their brief

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(ca. 4–6 h) exposure to neutral pH in the gastrointestinal tract as part of drug delivery systems, but are not stable enough to survive longer term exposure to alkaline pH, as in paint dispersions or other consumer products. Thus new, more flexible approaches are needed that tether the carboxyl to the polysaccharide by more hydrolytically robust links.

The bacterial exopolysaccharide curdlan is of particular interest because of its biomedical, solubility, and rheological properties (Edgar, 2014a, 2014b;). Curdlan is a linear, neutral glucan with (1 → 3)-β-glucosidic linkages, without branching or substituents (Harada, Masada, Hidaka, & Takada, 1966). Curdlan is soluble in alkaline media, though insoluble in water, and has enhanced solubility in organic solvents compared to many other natural polysaccharides. This solubility facilitates curdlan chemical reactions and processing. In recent years, curdlan and its derivatives have been investigated for biomedical or therapeutic uses, in part due to curdlan's low toxicity (Spicer, Goldenthal, & Ikeda, 1999).

Despite the challenges that (generally poorly reactive) polysaccharides present to chemo- and regioselective modification (Fox, Li, Xu, & Edgar, 2011), some recent progress has been made (Zheng, Gandour, & Edgar, 2013; Liu & Baumann, 2002; Kurita, Matsumura, Takahara, Hatta, & Shimojoh, 2011; Nakagawa et al., 2012; Zheng, Xu, & Edgar, 2015; Carvalho, Queda, Santos, & Marques, 2016; Liu, Liu, Esker, & Edgar, 2016; Bedini, Laezza, Parrilli, & Iadonisi, 2017; Liu & Edgar, 2017; Chien & Iwata, 2018). Perhaps the most selective reaction of polysaccharides that possess unsubstituted 6-OH groups is bromination using triphenylphosphine and *N*-bromosuccinimide (NBS), discovered by the Furuhata group (Furuhata, Koganei, Chang, Aoki, & Sakamoto, 1992; Tseng, Furuhata, & Sakamoto, 1995). This reaction is essentially perfectly selective within limitations of spectroscopic methods; hydrolysis to monosaccharides and chromatographic analysis confirms the absence of off-target bromination products (Furuhata et al., 1992). Several groups have leveraged this selective 6-bromination for regioselective C-6 amination of polysaccharides including cellulose (Liu et al., 2016; Matsui, Ishikawa, Kamitakahara, Takano, & Nakatsubo, 2005) and amylose (Cimecioglu, Ball, Kaplan, & Huang, 1994). Our group has exploited 6-bromo-6-deoxycurdlan, prepared by Furuhata bromination, through azide displacement followed by Staudinger reduction of the azide, or direct amine displacements of the 6-bromide, for regioselective synthesis of a series of 6-deoxy-6-(azido/amino/amido/ammonium) curdlan derivatives (Edgar, 2014a, 2014b; ; Zhang, Liu, & Edgar, 2016).

The iminophosphorane ylide that results from PPh_3 reduction of 6-azido-6-deoxy polysaccharides, including curdlan, has a highly nucleophilic, negatively charged nitrogen atom. Having proven that these curdlan ylides could be successfully acylated, alkylated with alkyl iodides, and reacted to aldehydes to form imines (which could be reduced to secondary amines), we realized that they might also be sufficiently good nucleophiles to effectively ring-open cyclic carboxylic acid anhydrides. We hypothesized that such ring opening would be successful and selective, and that the products would be curdlan 6-ω-carboxyalkanamides, where the carboxyl-containing chain was appended by a hydrolytically stable amide linkage. We further hypothesized that such derivatives could be highly valuable as amorphous solid dispersion (ASD) polymers, for enhancing solubility and oral bioavailability of otherwise poorly soluble drugs (Liu et al., 2014). The potential for these curdlan 6-ω-carboxyalkanamides as stable, carboxyl-containing, amphiphilic polymers for ASD is exciting, especially since they could likely be tailored to have high glass transition temperatures (T_g), desired solubility parameters, and other key ASD properties. They are also of interest beyond ASD; the likely hydrolytic stability of the amide linkage means that the tether to the key carboxyl group, which would provide the pH responsiveness, water-dispersibility, and specific interactions with other molecules, would survive a broad pH range. Therefore we could anticipate utility in higher pH dispersions like those typically used, e.g., in aqueous coating and adhesive formulations.

Herein, we describe our pursuit of a simple and efficient strategy for synthesizing ω-carboxyalkanamide-substituted polysaccharide derivatives from curdlan esters by reacting iminophosphorane ylides with cyclic anhydrides. We report their interesting physical properties, and describe initial screening as ASD polymers by crystallization inhibition experiments.

2. Materials and methods

2.1. Materials

Curdlan (degree of polymerization (DP) = 421, measured by size exclusion chromatography (SEC) of the per(phenylcarbamate) derivative) was from Wako Chemicals, vacuum-dried at 40 °C overnight before use. Lithium bromide (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 $CaCl_2$. *N,N*-Dimethylacetamide (DMAc, reagent grade, Fisher) was stored over 4 Å molecular sieves. Pyridine (anhydrous, 99%, AcroSeal), 4-dimethylaminopyridine (DMAP, Acros), triphenylphosphine (PPh_3, 99 + %, Sigma-Aldrich), succinic anhydride (99%, Sigma-Aldrich), glutaric anhydride (99%, Sigma-Aldrich), 3,3-dimethylglutaric anhydride (99%, Sigma-Aldrich), potassium bromide (Sigma-Aldrich), ethanol (HPLC grade, Fisher), molecular sieves (4 Å, Fisher) and regenerated cellulose dialysis tubing (MWCO 3500, Fisher) were used as received.

2.2. Measurements

^1H and ^{13}C NMR spectra were obtained on a Bruker Avance II 500 MHz spectrometer in DMSO-*d6* or DMF-*d7* at room temperature, employing 32, and 15,000 scans, respectively. Infrared spectra of samples as pressed KBr pellets were obtained on a Thermo Electron Nicolet 8700 instrument using 64 scans and 4 cm^{-1} resolution. SEC was performed on Agilent 1260 Infinity MultiDetector using DMAc with 0.05 M LiCl as mobile phase (50 °C) with 3 PLgel 10 μm mixed-B 300 × 7.5 mm columns in series. Data acquisition and analysis used Astra 6 software (Wyatt Technology Corporation, Goleta, CA). Monodisperse polystyrene standard (Mw ∼21k, polydispersity index (PDI) ∼1.02) was run first in every sample series for calibration and confirmation. C, N contents were determined by Micro Analysis Inc. using a Perkin Elmer 2400 II analyzer, while bromine content was determined with a thermal conductivity detector. DS values were determined by ^1H NMR spectroscopy, according to the following equations:

$$\mathrm{DS(omega - carboxypropionamide)} = \frac{7I_{SA-CH_2}}{4}$$

$I_{SA\text{-}CH2}$: the signal from methylene groups of succinyl moieties

$$\mathrm{DS(omega - carboxybutyramide)} = \frac{7I_{CH2}}{6} - 1$$

I_{CH2}: the signal from methylene groups of glutaryl moieties

$$\mathrm{DS(omega{-}3{,}3 - dimethyl{-}carboxybutyramide)} = \frac{7I_{CH3}}{6}$$

I_{CH3}: the signal from methyl groups of 3,3-dimethylglutaryl moieties

$$\mathrm{DS(Ac)} = \frac{7I_{Ac\ CH_3}}{3}$$

I_{AcCH3}: the signal from methyl groups of acetyl moieties

2.3. Synthesis of 6-bromo-6-deoxy-curdlan

Curdlan dissolution in DMAc/LiBr was adapted from a previous method for cellulose dissolution (Edgar, Arnold, Blount, Lawniczak, & Lowman, 1995). Ph_3P (25.96 g, 4 equiv/AGU) and NBS (17.58 g, 4