Water-Soluble Co-polyelectrolytes by Selective Modification of Cellulose Esters

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Abstract

Cellulose-based materials are well-suited for biomedical uses, because of their abundance, renewable nature, biodegradability, and relatively low cost. However, the set of commercially available cellulose esters and ethers is limited in number and diversity, and contains no cationically charged cellulose esters. Herein we report a simple, efficient strategy for synthesizing cationic, water-soluble co-polyelectrolytes from commercial, hydrophobic, renewable-based cellulose esters. Cellulose acetate (degree of substitution (DS) 1.78, CA320S), was the exemplary starting material for preparing these cationic polyelectrolytes by a reaction sequence of phosphine-catalyzed bromination and subsequent displacement by an aromatic amine, affording high reaction conversions. We show that these modification techniques can be carried out with essentially complete regio- and chemoselectivity, proceeding in the presence of multiple ester groups, yet preserving those groups. Availability of these novel polysaccharide-based electrolytes starting from uncharged, commercial, inexpensive cellulose esters may open up multiple new application areas, including in several aspects of gene or drug delivery.

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25 1. Introduction

26 Polysaccharides, one of the most abundant and diverse families of natural polymers, exhibit
27 an incredibly wide range of natural functions including structural reinforcement (Caffall &
28 Mohnen, 2009), energy storage (Slock & Stahly, 1974), modification of aqueous rheology
29 (Lapasin & Prici, 1995), and communication (Sakurai & Shinkai, 2000). Native cellulose is a
30 homopolymer ($\rightarrow 4\text{-}\beta\text{-D-GlcP-1}\rightarrow$) of the monosaccharide D-glucopyranose, without
31 branching or substituents (Dubin, Bock, Davies, Schulz & Thies, 1994). Its derivatives
32 dominate commercial uses and sales of polysaccharide derivatives (Edgar et al.,
33 2001). Application of native cellulose as a sustainable material is impeded by its insolubility in
34 common solvents including water, poor dimensional stability, and lack of
35 thermoplasticity. To increase its functionality and utility, researchers have tailored the chemical
36 and physical properties of cellulose using a variety of chemical modification techniques,
37 resulting in a relatively small number of commercial derivatives. Hundreds of millions of
38 kilograms of cellulose and its derivatives are sold annually and used for numerous
39 applications including coatings, optical films, pharmaceuticals, and composite materials
40 (Heinze, 2005).

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42 Esters of cellulose, including cellulose nitrate and its organic esters, have been among its
43 most important and useful derivatives (Edgar et al., 2001; Klemm, Heublein, Fink & Bohn,
44 2005). More recently, the small set of cellulose organic esters that could be synthesized
45 practically given the limitations of conventional esterification has been expanded (Fox, Li,
46 Xu & Edgar, 2011; Dong & Edgar, 2015; Meng, Matson & Edgar, 2014a, b; Xu & Edgar,
47 2012; Zhang, Zheng, Kuang & Edgar, 2014; Zheng, Gandour & Edgar, 2013a, b), assisted

48 by the development of cellulose solvents (Dawsey& McCormick, 1990; Östlund, Lundberg,
49 Nordstierna, Holmberg & Nydén, 2009; Zhu et al, 2006).Regioselective ester synthesis has
50 afforded access to new structures and properties, e.g. through selective protection and
51 deprotection (Gomez, Erler & Klemm, 1996; Hall & Horne, 1973; Hearon, Hiatt & Fordyce,
52 1943; Heinze, Röttig & Nehls, 1994; Heinze, Pfeifer, Sarbova & Koschella, 2011; Helferich
53 & Köster, 1924; Honeyman, 1947; Klemm & Stein, 1995; Koschella, Heinze & Klemm,
54 2001; Koschella, Fenn & Heinze, 2006; Yin, Koschella & Heinze, 2009),oxidation at the
55 primary alcohol groups(Bragd, van Bekkum & Besemer, 2004; Fukuzumi, Saito, Iwata,
56 Kumamoto & Isogai, 2009; Isogai & Kato, 1998; Saito & Isogai, 2004; Saito, Kimura,
57 Nishiyama & Isogai, 2007),and Staudinger reactions(Fox & Edgar, 2012; Liu &Edgar, 2015).
58 In 1992, Furuhata et al. reported a simple and powerful method for regioselective
59 halogenation at the C-6 position (Furuhata, Koganei, Chang, Aoki & Sakamoto, 1992) by
60 dissolving cellulose in DMAc/lithium bromide, thenadding triphenylphosphine (Ph₃P) and
61 N-bromosuccinimide (NBS). An advantage versus frequently-described cellulose tosylation is
62 that Furuhata bromination is virtually quantitative and completely selective for C-6, in
63 contrast with a significant amount of off-target reaction with tosylation (Cimecioglu, Ball,
64 Kaplan & Huang, 1994; Gericke et al., 2012; Koschella & Heinze, 2003; Pereira & Edgar,
65 2014; Zhang & Edgar, 2014).6-Deoxy-6-bromocellulose has proven to be a versatile
66 intermediate for further modification of cellulose. Thiols can effectively displace the 6-
67 bromide,thereby regioselectively attaching different pendent functional groups, such as
68 carboxylic acids to form anionic cellulose derivatives(Aoki, Furuhata, Saegusa, Nakamura &
69 Sakamoto, 1996)orreaction withsodium sulfite can afford water-soluble 6-sulfonate
70 derivatives (Furuhata & Ikeda, 1999).

Cationic cellulose derivatives have recently received increasing attention, since they are capable of binding electrostatically with anionic biomolecules including nucleic acids and certain proteins to produce therapeutically useful polyelectrolyte complexes (Scranton, Rangarajan & Klier, 1995). Compared to synthetic cationic polymers, cationic cellulose derivatives may in some cases be more attractive candidates for therapeutic uses because they frequently are more biocompatible and biodegradable, and have low immunogenicity (Samal et al., 2012). Recently we have prepared cationic derivatives of cellulose and other glucans that possess free 6-OH groups by reacting trialkylamines with 6-bromo-6-deoxyglucans, for example 6-bromo-6-deoxy-2,3-diacetyl-cellulose, which is generated from native cellulose by Furuhashi bromination and *in situ* peracylation (Marks, Fox & Edgar, 2016). However, we found that such nucleophilic bromide displacements by trialkylamines are quite difficult to drive to high reaction conversion, possibly due to developing charge-charge repulsion in the increasingly cationic product (Liu, Liu, Esker & Edgar, 2016; Marks, Fox & Edgar, 2016; Zhang, Liu & Edgar, 2016). We have also observed that aromatic amines (e.g., pyridine and 1-methylimidazole) are more efficient nucleophiles in such displacement reactions, affording high DS values of the cationic substituents (Liu, Liu, Esker & Edgar, 2016). These cationic cellulose derivatives exhibit surprisingly high thermal stability and good water solubility, and are capable of binding irreversibly to hydrophilic and anionic surfaces. However, all of these cationic cellulose ionomers synthesized using phosphine-catalyzed bromination and aromatic amine displacements were derived from low degree of polymerization (DP) microcrystalline cellulose (MCC). MCC is popular for cellulose solution methods, since its solutions are less viscous and more easily managed than those that result from high DP dissolving pulp. However, this low starting DP restricts the utility of its derivatives for plastics and other applications where high DP is needed. Also, since 100%

conversion of the 6-bromo groups of 6-bromo-6-deoxycellulose to cationic substituents is very difficult to achieve, the products still contain 6-bromide residues, which could be alkylating sites *in vivo* (reacting for example with endogenous proteins and peptides) and therefore could lead to polymer toxicity.

Thus, there could be considerable advantage if one could start with high DP commercial esters, so long as they contain substantial DS of residual 6-OH groups. Such starting materials could in theory afford quantitative conversion of the resulting 6-bromo groups to, e.g., ammonio substituents (since positive charges would be on average more widely separated), and provide water- and organic-soluble, high DP products useful in a wider variety of applications. However, there was considerable reason to be concerned about whether bromination of such derivatives would be effective. Would the simultaneous presence in the brominated cellulose esters of alkyl halides and hydroxyl groups lead to crosslinking via bromide displacement? Would the ester groups lead to undesired side reactions (e.g. deacylation catalyzed by the trialkylamine base)? Would the products have sufficient DS of ammonio groups to afford the desired properties?

We hypothesize that Furuhashi bromination of cellulose esters containing a sufficient concentration of free 6-OH groups will afford selectively, partially brominated 6-bromo-6-deoxy cellulose ester derivatives. We further hypothesize that displacement of these 6-bromo groups by tertiary amines will afford, in high conversion, 6-ammonio-6-deoxycellulose ester polyelectrolytes that will have high DP, and good solubility in both water and organic solvents. In this work, we attempt to confirm these hypotheses by applying phosphine-catalyzed bromination and subsequent aromatic amine displacements to commercial

cellulose esters, in order to prepare cellulose-based sustainable materials for advanced technologies. We selected a commercial cellulose acetate with high DS(OH), cellulose acetate (DS(Ac) 1.78, *vide infra*), as substrate for our planned phosphine-catalyzed bromination. We report attempts to functionalize the resulting derivative by azide and aromatic amine displacements to prepare cellulose-based *N*-containing copolymers, including polyelectrolytes.

2. Experimental

2.1. Materials

Cellulose acetate (CA320S, DS(Ac) 1.78, DS(6-OH) 0.49 (measured using perpropionylated sample by ^1H nuclear magnetic resonance (NMR)), $M_w = 45.3$ kDa (measured by SEC), Eastman Chemical Company) was dried under vacuum at 50 °C overnight before use. *N*-Bromosuccinimide (NBS, 99%, Acros) was recrystallized from boiling water and dried for two days under reduced pressure over anhydrous calcium chloride. 4-(Dimethylamino)pyridine (DMAP, Sigma-Aldrich), triphenylphosphine (Ph_3P , Sigma-Aldrich), sodium azide (NaN_3 , Fisher), pyridine (anhydrous, 99+%, AcroSeal), imidazole (99+%, Sigma-Aldrich), 1-methylimidazole (99+%, Sigma-Aldrich), methyl iodide (Sigma-Aldrich), and potassium bromide (KBr, Sigma-Aldrich) were used as received. Methanol, ethanol and *N*-methyl-2-pyrrolidone (NMP) were from Fisher Scientific, Pittsburgh, PA and used as received. *N*, *N*-Dimethylacetamide (DMAc, Fisher), propionic anhydride (Sigma-Aldrich), and dimethyl sulfoxide (DMSO, Acros) were kept over 4 Å molecular sieves under dry nitrogen until use. Regenerated cellulose dialysis tubing (3500 g/mol molecular weight cut-off (MWCO)) was purchased from Fisher and used as received.

2.2. Measurements

¹H and ¹³C NMR spectra were obtained on a Bruker AVANCE II 500 MHz spectrometer in DMSO-*d*₆ at room temperature or 50 °C. 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. Size exclusion chromatography (SEC) was performed on Agilent 1260 Infinity MultiDetector SEC using DMAc with 0.05 M LiCl as the mobile phase (50 °C) with 3 PLgel 10 μm mixed-B 300 × 7.5 mm columns in series. Data acquisition and analysis was conducted using 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 the purpose of calibration and confirmation. Carbon, nitrogen, and bromine contents were determined by Micro Analysis Inc. using a Perkin Elmer 2400 II analyzer. Carbon and nitrogen contents were measured by flask combustion followed by ion chromatography, and bromine content was determined with a thermal conductivity detector. Zeta potentials (Zetasizer NanoZS, Malvern Instruments) were measured at 25°C. DS values were determined by means of ¹H NMR spectroscopy, according to the following equations, respectively.

$$DS_{Ac} = \frac{7I_{CH_3}}{3I_{cellulose \ backbone}}$$

$$DS_{Pyr^+} = \frac{7I_{CH-ring}}{5I_{cellulose \ backbone}}$$

$$DS_{MeIM^+} = \frac{7}{\frac{3I_{cellulose \ backbone} + CH_3}{I_{CH-ring}} - 3}$$

$$DS_{IM} = \frac{7I_{CH-ring}}{3I_{cellulose \ backbone}}$$

2.3. Perpropionylation of CA320S

Cellulose acetate (CA320S) was propionylated for easier NMR analysis using methods first described by the Heinze group and adapted from previous studies (Liebert, Hussain & Heinze, 2005; Xu, Li, Tate & Edgar, 2011; Zheng, Gandour & Edgar, 2013a). 4-(Dimethylamino)pyridine (15 mg) and propionic anhydride (4 mL) were added to a solution of CA320S (300 mg) in pyridine (4 mL) at 80 °C and stirred for 24 h, then the cooled reaction solution was added slowly to ethanol. The precipitated product was collected by filtration and was washed several times with ethanol. The crude product was redissolved in chloroform (5 mL), reprecipitated into ethanol (150 mL), and washed several times with ethanol. The resulting material was dried under vacuum to yield the perpropionylated CA320S.

2.4. Regioselective bromination of CA320S

In a 100 mL three-necked round-bottom flask, 1.00 g CA320S (4.22 mmol) was dissolved in 40 mL of DMAc. Ph_3P (3 equiv per AGU, 12.66 mmol, 3.32 g) and NBS (3 equiv per AGU, 12.66 mmol, 2.25 g) were separately dissolved in 10 mL portions of DMAc. The Ph_3P solution was added dropwise to the CA320S solution, followed by the dropwise addition of the NBS solution. The resulting solution was heated to 70 °C under nitrogen for 1 h while stirring. It was then cooled and added slowly to 1 L of a 50:50 (v/v) mixture of methanol and deionized water to precipitate the product, followed by filtration. The precipitate was then twice redissolved in acetone, followed by precipitation in ethanol, then was dried overnight in a vacuum oven at 50 °C, affording (6-bromo-6-deoxy)- α -(6-O-acetyl)-CA 320S (6-BrCA320S) (992 mg, 3.59 mmol). Yield: 85%. ^{13}C NMR (500 MHz, CDCl_3): 21.06 (O-(C=O)- CH_3), 33.52 ($\text{C}-6-\text{Br}$), 62.87 ($\text{C}-6'-\text{O}-\text{Ac}$) 71.84-78.46 (C-2, C-3, C-4 and C-5),

100.09 (C-1), 103.47 (C-1'), 170.85 (O-(C=O)-CH₃). Elemental analysis: %C 42.13, %H 4.69, %N None Found, %Br 14.21 (Theoretical: (DS(Br) 0.49) %C 41.69, %H 6.44 %, N 0%, Br 14.18); DS by elemental analysis: DS(Br) 0.49.

2.5. Synthesis of (6-azido-6-deoxy)- α -(6-O-acetyl)-CA320S (6-N₃CA320S)

In a 100 mL three-necked round-bottom flask, 250 mg 6-BrCA320S (0.9 mmol) was dissolved in 10 mL of DMSO. Sodium azide (3 equiv per AGU, 2.7 mmol, 176 mg) was added to the flask. The solution was heated to 80 °C and stirred at that temperature for 24 h under nitrogen. The cooled solution was then transferred to dialysis tubing. After 3 days of dialysis against ethanol and 3 days of dialysis against deionized water, the solution was freeze-dried to yield 6-N₃CA320S (207 mg, 0.79 mmol). Yield: 87%. ¹³C NMR (500 MHz, DMSO-*d*₆): 21.02 (O-(C=O)-CH₃), 50.41 (C-6-Br), 62.86 (C-6'-O-Ac), 77.00-81.00 (C-2, C-3, C-4 and C-5), 99.75 (C-1), 103.05 (C-1'), 169.86 (O-(C=O)-CH₃). Elemental analysis: %C 45.13, %H 5.30, %N 7.86, %Br None Found (Theoretical: (DS(N₃) 0.49) %C 44.70, %H 6.90, %N 7.99, %Br 0). DS by elemental analysis: DS(N₃) 0.49.

2.6. Synthesis of (6-pyridinio-6-deoxy)- α -(6-O-acetyl)-CA320S (6-PyrCA320S)

In a 100 mL three-necked round-bottom flask, 250 mg 6-BrCA320S (0.9 mmol) was dissolved in 10 mL of DMSO. Pyridine (1.42 g, 18 mmol, 20 equiv per AGU) was added to the flask. The solution was heated to 80 °C and stirred at that temperature for 48 h under nitrogen. The cooled solution was then transferred to dialysis tubing. After dialysis (3 days against ethanol, then 3 days against deionized water), the solution was freeze-dried to yield 6-PyrCA320S (243 mg, 0.80 mmol). Yield: 89%. ¹H NMR (500 MHz, DMSO-*d*₆): 1.84-2.07 (O-(C=O)-CH₃), 3.00-6.00 (cellulose backbone), 8.22 (N-CH=CH-CH), 8.67 (N-

CH=CH-CH), 8.95 (N-CH≡CH-CH); ¹³C NMR (500 MHz, DMSO-*d*₆): 20.65 (O-(C=O)-
CH₃), 60.98 (C-6), 62.85 (C-6'), 71.00-77.00 (C-2, C-3, C-4 and C-5), 99.73 (C-1), 103.16 (C-
1'), 128.26 (N-CH=CH-CH), 146.41 (N-CH=CH-CH and N-CH=CH-CH), 169.86 (O-
(C=O)-CH₃). DS by ¹H NMR: DS(Pyr⁺) 0.41, DS(Ac) 1.82. Elemental analysis: %C 44.09,
%H 4.91, %N 1.91, %Br 8.62 (Theoretical: (DS(Pyr⁺) 0.49) %C45.65, %H 6.41,%N 2.21,
%Br12.44). DS by elemental analysis: DS(Pyr⁺) 0.42.

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216 **2.7. Synthesis of (6-(1-methyl-3-imidazolio)-6-deoxy)-*co*-(6-*O*-acetyl)-CA320S (6-** 217 **MeIMCA 320S**

6-BrCA320S(250 mg, 0.9 mmol) was dissolved in 10 mL of DMSO in a 100 mL three-
necked round-bottom flask. 1-Methylimidazole (1.47 g, 18 mmol, 20 equiv per AGU) was
added to the flask. The solution was heated to 80 °C and stirred at that temperature for 48 h
under nitrogen. The cooled solution was transferred to dialysis tubing. After dialysis (3 days
against ethanol, then 3 days against deionized water), the solution was freeze-dried to yield 6-
MeIMCA320S (255 mg, 0.81 mmol). Yield: 90%. ¹H NMR (500 MHz, DMSO-*d*₆): 1.86-2.07
(O-(C=O)-CH₃), 3.00-6.00 (cellulose backbone and N-CH₃), 7.69-7.82 (N-CH=CH-N-
CH₃ and N-CH=CH-N-CH₃), 9.14 (N=CH-N-CH₃); ¹³C NMR (500 MHz, DMSO-*d*₆):
20.64 (O-(C=O)-CH₃), 36.23 (N-CH₃), 49.55 (C-6), 63.01 (C-6'), 71.00-80.00 (C-2, C-3, C-4
and C-5), 99.80 (C-1), 103.57 (C-1'), 123.50 (N-CH=CH-N-CH₃), 123.70 (N-CH=CH-N-
CH₃), 137.69 (N=CH-N-CH₃), 169.82 (O-(C=O)-CH₃). DS by ¹H NMR: DS(MeIM⁺) 0.46,
DS(Ac) 1.70. Elemental analysis: %C 44.28, %H 5.32, %N 4.19, %Br 6.63 (Theoretical:
(DS(MeIM⁺) 0.49) %C 47.53, %H 6.54, %N 4.34, %Br 12.39). DS by elemental analysis:
DS(MeIM⁺) 0.47.

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2.8. One pot synthesis of 6-MeIMCA320S

CA320S (1.00 g, 4.22 mmol) was dissolved in 40 mL of DMAc in a 100 mL three-necked round-bottom flask. Ph₃P (3.32 g, 12.66 mmol, 3 equiv per AGU) and NBS (2.25 g, 12.66 mmol, 3 equiv per AGU) were separately dissolved in 10 mL portions of DMAc. The Ph₃P solution was added dropwise to the CA320S solution, followed by the dropwise addition of the NBS solution. The resulting solution was heated to 70 °C under nitrogen for 1 h while stirring. 1-Methylimidazole (20 equiv per AGU) was added to the flask. The solution was heated to 80 °C and stirred at that temperature for 48 h under nitrogen. The cooled solution was transferred to dialysis tubing. After dialysis (3 days against ethanol, then 3 days against deionized water), the solution was freeze-dried to yield 6-MeIMCA 320S (990 mg, 3.19 mmol). Yield: 88%. ¹H NMR (500 MHz, DMSO-*d*₆): 1.86-2.07 (O-(C=O)-CH₃), 3.00-6.00 (cellulose backbone and N-CH₃), 7.69-7.82 (N-CH=CH-N-CH₃ and N-CH=CH-N-CH₃), 9.14 (N=CH-N-CH₃); ¹³C NMR (500 MHz, DMSO-*d*₆): 20.66 (O-(C=O)-CH₃), 36.27 (N-CH₃), 49.59 (C-6), 63.04 (C-6'), 71.00-80.00 (C-2, C-3, C-4 and C-5), 99.80 (C-1), 103.57 (C-1'), 123.68 (N-CH=CH-N-CH₃ and N-CH=CH-N-CH₃), 138.50 (N=CH-N-CH₃), 170.86 (O-(C=O)-CH₃). DS by ¹H NMR: DS(MeIM⁺) 0.41, DS(Ac) 1.70.

2.9. Synthesis of (6-imidazolyl-6-deoxy)-*co*-(6-*O*-acetyl)-CA320S (6-IMCA320S) and quaternization of 6-IMCA320S

In a 100 mL three-necked round-bottom flask, 6-BrCA320S (250 mg, 0.9 mmol) was dissolved in 10 mL of DMSO. Imidazole (1.84 g, 30 equiv per AGU) was added to the flask and dissolved. The solution was heated to 80 °C and held at that temperature for 48 h under nitrogen with stirring. The cooled reaction solution was added to dialysis tubing, followed by dialysis (3 days against ethanol, then 3 days against deionized water). The retentate was finally

dried under vacuum overnight to yield 6-IMCA320S (192 mg, 0.71 mmol). Yield: 79%. ^1H NMR (500 MHz, $\text{DMSO}-d_6$): 1.70-2.30 ($\text{O}-(\text{C}=\text{O})-\underline{\text{CH}}_3$), 3.00-6.00 (cellulose backbone), 7.60-7.80 ($\text{N}-\text{CH}=\underline{\text{CH}}-\text{N}$ and $\text{N}-\underline{\text{CH}}=\text{CH}-\text{N}$), 8.80-9.10 ($\text{N}-\underline{\text{CH}}=\text{N}$); ^{13}C NMR (500 MHz, $\text{DMSO}-d_6$): 21.03 ($\text{O}-(\text{C}=\text{O})-\underline{\text{CH}}_3$), 46.90 (C-6), 62.88 (C-6'), 71.00-77.00 (C-2, C-3, C-4 and C-5), 99.72 (C-1), 103.17 (C-1'), 120.50 ($\text{N}-\text{CH}=\underline{\text{CH}}-\text{N}$), 128.78 ($\text{N}-\underline{\text{CH}}=\text{CH}-\text{N}$), 138.35 ($\text{N}-\underline{\text{CH}}=\text{N}$), 169.89 ($\text{O}-(\underline{\text{C}}=\text{O})-\text{CH}_3$). DS by ^1H NMR: DS(IM) 0.49, DS(Ac) 1.77. Elemental analysis: %C 49.41, %H 5.63, %N 4.82, %Br None Found(Theoretical: (DS(IM) 0.49) %C 49.35,%H 7.51, %N 5.18 %Br 0). DS by elemental analysis: DS(IM) 0.49.

In a 25 mL round-bottom flask, 6-IMCA320S (100 mg, 0.37 mmol) was dissolved in 10 mL of NMP. Methyl iodide (10 equiv per AGU) was added to the flask. The solution was heated to 50 °C and held at that temperature for 48 h under reflux with stirring. The cooled reaction solution was added to dialysis tubing, followed by dialysis (3 days against ethanol, then 3 days against deionized water). The retentate was finally dried under vacuum overnight to yield 6-MeIMCA320S (101 mg, 0.32 mmol). Yield: 86%. ^1H NMR (500 MHz, $\text{DMSO}-d_6$): 1.86-2.07 ($\text{O}-(\text{C}=\text{O})-\underline{\text{CH}}_3$), 3.00-6.00 (cellulose backbone and $\text{N}-\underline{\text{CH}}_3$), 7.69-7.82 ($\text{N}-\underline{\text{CH}}=\text{CH}-\text{N}-\text{CH}_3$ and $\text{N}-\text{CH}=\underline{\text{CH}}-\text{N}-\text{CH}_3$), 9.14 ($\text{N}=\underline{\text{CH}}-\text{N}-\text{CH}_3$); ^{13}C NMR (500 MHz, $\text{DMSO}-d_6$): 20.56 ($\text{O}-(\text{C}=\text{O})-\underline{\text{CH}}_3$), 36.17 ($\text{N}-\underline{\text{C}}\text{H}_3$), 49.49 (C-6), 63.08 (C-6'), 71.00-80.00 (C-2, C-3, C-4 and C-5), 99.83 (C-1), 103.58 (C-1'), 123.82 ($\text{N}-\text{CH}=\underline{\text{CH}}-\text{N}-\text{CH}_3$ and $\text{N}-\underline{\text{CH}}=\text{CH}-\text{N}-\text{CH}_3$), 138.58 ($\text{N}=\underline{\text{C}}\text{H}-\text{N}-\text{CH}_3$), 170.94 ($\text{O}-(\underline{\text{C}}=\text{O})-\text{CH}_3$). DS by ^1H NMR: DS(N-methyl) 0.39, DS(Ac) 1.70.

3. Results and discussion

In this work, a commercial cellulose acetate (CA320S) was selected as starting material because it has relatively high molecular weight and, most crucially, contains a high DS(OH). Because cellulose ester synthesis typically involves peracetylation followed by back-hydrolysis to the desired DS(acyl), CA 320S should have a relatively high DS(6-OH) (since the primary alcohol acetate has wider approach angles and therefore should be hydrolyzed most rapidly). In order to measure the acetyl content at each position, CA320S was perpropionylated by reacting with propionic anhydride in the presence of pyridine and DMAP. The acetyl singlets in the ^1H NMR spectrum were readily assigned based on HMBC and other NMR methods (Zheng, Gandour & Edgar, 2013a), and thus readily quantified by the ratios of their integrals to that of the backbone protons. By this method (Figure S1), DS(Ac) for CA 320S calculated is 1.78, which is consistent with previous reports (Zheng, Gandour & Edgar, 2013a). DS(6-Pr) calculated from ^1H NMR is 0.49. Therefore, DS(6-Ac) is 0.51 and DS(6-OH) is 0.49 (see Table S1 for other positional DS data by this method).

Scheme 1. Reaction scheme for conversions of CA320S to 6- $\text{N}_3\text{CA320S}$, 6-PyrCA320S and 6-MeIMCA320S.

3.1. 6-BrCA320S

We attempted to brominate CA320S using a procedure adapted from Furuhashi et al. (Furuhashi, Koganei, Chang, Aoki & Sakamoto, 1992), reacting with Ph_3P and NBS in DMAc at 70 °C for 1 h (Scheme 1). The product ^{13}C NMR spectrum (Figure 1) provides confirmation that the desired C-6 bromination has cleanly occurred. A new resonance appears at 37 ppm, consistent with bromo-substituted C-6 (Fox & Edgar, 2012). Based on previous

reports(Liu, Liu, Esker & Edgar, 2016; Zhang, Liu & Edgar, 2016), the resonance at 63 ppm is from 6-carbons bearing acetyl groups. Resonances at 130 and 135 ppm indicate that the product contains small proportions of Ph_3P and its oxide; as in our previous studies, it was difficult to completely remove phosphine impurities from the initial brominated product(Fox & Edgar, 2012; Zhang & Edgar, 2014). Satisfyingly, $\text{DS}(\text{Br})$ calculated from elemental analysis was 0.49. Since the $\text{DS}(\text{6-OH})$ of CA320S was measured as 0.49 by integration of the 6-propionate methyl in the perpropionylated product, C-6 bromination is quantitative. The resulting 6-BrCA320S exhibits good solubility in commonly used organic solvents such as DMAc, DMSO or DMF, indicating that little or none of the feared crosslinking reaction had taken place. The ability to carry out the desired bromination cleanly and quantitatively on a cellulose ester was a promising start.

Figure 1. ^{13}C NMR spectrum of 6-BrCA320S.

3.2. Azide Displacement

The key remaining questions were whether the 6-bromo substituents of 6-BrCA320S were sufficiently susceptible to nucleophilic displacement, and whether the reaction would be free of side reactions like base-catalyzed deacylation. We first explored displacement by the highly nucleophilic azide. The Edgar and Kaplan groups, for example, have previously demonstrated that bromide displacement by azide was efficient on 6-bromo-6-deoxypolysaccharide derivatives of native cellulose, amylose, pullulan, and curdlan (Cimecioglu, Ball, Kaplan & Huang, 1994; Fox & Edgar, 2012; Pereira & Edgar, 2014; Zhang & Edgar, 2014). In addition, azide is a useful handle for click chemistry (Liebert, Hänsch & Heinze, 2006) or Staudinger ligations (Gattás-Asfura & Stabler, 2009), permitting complex and useful polysaccharide functionalization. In the event, reaction of 6-BrCA320S with NaN_3 in DMSO at 80 °C for 24 h (Scheme 1) was successful. By ^{13}C NMR (Figure S2), the C-Br peak at 37 ppm was absent in the product, while a new peak at 50 ppm was assigned as the C- N_3 resonance. The product FTIR spectrum (Figure S3) is conclusive, with a strong azide absorption at 2110 cm^{-1} and a carbonyl absorption from the ester at 1760 cm^{-1} , supporting successful azide displacement without apparent loss of ester groups. The absorption at 3500 cm^{-1} results from the unreacted (as expected) secondary OH groups at C-2 and C-3, and possibly from trace moisture in the sample. Elemental analysis indicated DS(N_3) 0.49, with no bromine content found, supporting quantitative overall conversion of the original 6-OH groups to 6- N_3 groups. Successful synthesis of azido-substituted cellulose ester co-polymers creates a gateway to a panoply of useful, regioselectively functionalized cellulose-based amines and amides (Zhang & Edgar, 2014).

3.3. Cationic Copolymer Electrolytes (6-PyrCA320S and 6-MeIMCA320S) Derived from 6-BrCA320S

We first tested our hypothesis that the lower DS(Br) of 6-BrCA320S would permit more complete displacement by aromatic amines by reacting pyridine with 6-BrCA320S in DMSO at 80 °C for 48 h (Scheme 1). In the product ^{13}C NMR spectrum (Figure S4), the peak for 6-bromide at 37 ppm was absent, while the new peak at 61 ppm is consistent with pyridinio-substituted C-6. The ^1H NMR spectrum also supported product identification as 6-PyrCA320S (Figure 2); new resonances at 8-10 ppm are assigned to the aromatic protons of the added pyridinium substituent. DS(Pyr $^+$) calculated from ^1H NMR is 0.41, implying that the reaction conversion is 84%. The conversion is indeed higher than from pyridine displacement on 6-bromo-6-deoxy MCC (75%) (Liu, Liu, Esker & Edgar, 2016). Still incomplete conversion may result from the fact that pyridine is a weak base and thus not a very good nucleophile for $\text{S}_{\text{N}}2$ displacement. Pyridine acting as a base could in theory have catalyzed deacylation of some cellulose ester groups. However, DS(Ac) was measured by ^1H NMR to be 1.75 (vs. the starting CA 320S DS(Ac) of 1.78), indicating that little or no deacetylation occurred during phosphine-catalyzed bromination and pyridine displacement.

Figure 2. ^1H NMR spectrum for 6-PyrCA320S.

In order to improve the conversion, we explored displacement by the more basic and nucleophilic 1-methylimidazole. Reaction of 6-BrCA320S with 1-methylimidazole in DMSO at 80 °C for 48 h (Scheme 1) afforded a product whose ^{13}C NMR spectrum (Figure S5) shows a new resonance for the imidazolium-substituted C-6 around 50 ppm; no trace of the brominated carbon (37 ppm) remains. The product ^1H NMR spectrum (Figure 3) shows new resonances at 7.5-9.5 ppm, assigned to the aromatic protons of the 1-methylimidazolium substituent, and a sharp and strong peak at 4 ppm from the protons of the *N*-methyl group. DS(MeIM $^+$) calculated from ^1H NMR is 0.46, implying that the reaction conversion is 94%. This is improved vs. pyridine displacement (84%), consistent with the higher nucleophilicity of the imidazole, leaving very little covalently bonded bromide in the product. It is also improved vs. that observed from fully 6-brominated MCC (83%), consistent with the hypothesis that developing charge density (higher in 6-BrMCC) limits conversion (Liu, Liu,

Esler & Edgar, 2016). The DS(Ac) of 6-MeIMCA320S calculated from ^1H NMR is 1.70, again indicating that there is no significant deacetylation during the reaction.

Figure 3. ^1H NMR spectrum for 6-MeIMCA320S.

3.4. One-Pot Synthesis of 6-MeIMCA320S

Having achieved a high degree of conversion to a cationic polyelectrolyte using 1-methylimidazole as nucleophile, we wondered whether bromination and aromatic amine displacement could be carried out in one pot without isolation of the intermediate in order to enhance efficiency. To test this possibility, CA320S was first reacted with NBS and Ph_3P in DMAc (70 $^\circ\text{C}$, 1 h). Then 1-methylimidazole was added to this solution, and temperature was increased to 80 $^\circ\text{C}$ for 48 h. The ^{13}C NMR spectrum (Figure 4) of the product isolated from this one-pot sequence had a new resonance for the imidazolium-substituted C-6 around 50 ppm, with no trace of the brominated carbon (37 ppm). Both ^1H and ^{13}C NMR spectra were

essentially identical to those of the product from “2-pot” synthesis, indicating that the one-pot reaction was successful. DS(MeIM⁺) calculated from ¹H NMR spectrum of the one-pot sequence product was 0.41, slightly lower conversion (84%) than in the 2-pot sequence.

Figure 4. ¹³C NMR spectrum for one-pot synthesized 6-MeIMCA320S.

3.5. 6-IMCA320S and Quaternization of 6-IMCA320S

Successful 1-methylimidazole displacement at rather high conversion was encouraging, but conversion was still short of 100%. This was perhaps due to random instances along the CA-320S chain where multiple successive monosaccharides had free 6-OH groups, thus resulting in charge repulsion between the eventual neighboring cationic groups. On the other hand, displacement by azide, since it did not generate charged groups, was quantitative. Therefore, it was of particular interest for us to study the reaction between 6-BrCA320S and imidazole itself, since such displacement would create no charge and thus might be expected to occur

in quantitative fashion, eliminating all 6-bromide groups. Note that, unlike 1-alkylimidazoles, imidazole contains two nucleophilic nitrogen atoms. If imidazole can be efficiently appended to C-6 of cellulose via S_N2 bromide displacement, the other basic nitrogen of imidazole could then be used as a nucleophile for incorporation of other interesting functional groups. In particular, we could generate cationic charge e.g. by methylation, or zwitterionic derivatives by reaction, e.g., with propane sulfone (Liu, Liu, Esker & Edgar, 2016), and these derivatives would not possess any undesirably reactive alkyl bromide groups. It has also been demonstrated that the imidazole group can be involved in metal-ligand interactions with metal ions, e.g. zinc ions (Mozhdehi, Ayala, Cromwell & Guan, 2014).

There was some concern that reaction with difunctional imidazole could cause cross-linking by further reaction of the second nucleophilic nitrogen with 6-bromo-6-deoxy substituents on other cellulose chains. To avoid such crosslinking, excess imidazole was employed to react with 6-BrCA in DMSO (80°C, 48 h, Scheme 2). The product ^{13}C NMR spectrum (Figure S6) confirmed the desired product identity; for example, the imidazole-substituted C-6 appeared at 48 ppm, shifted downfield from 32 ppm in the brominated starting material. No trace of the starting material brominated carbon remains in the product spectrum. ^1H NMR spectroscopy (Figure 5) also supported product identity as 6-IMCA320S; new peaks at 8-10 ppm are assigned to the aromatic protons of the incorporated imidazole substituent. Elemental analysis indicated no residual bromine in the product, confirming quantitative nucleophilic displacement by imidazole (DS(IM) 0.49, confirmed by ^1H NMR). 6-IMCA exhibits good solubility in common organic solvents including DMSO and DMF, whereas the uncharged polymer is insoluble in water. This good solvent solubility along with the

spectroscopic evidence support the notion that no cross-linking has occurred during 6-IMCA synthesis.

Scheme 2. Reaction scheme for conversion of 6-BrCA320S to 6-IMCA320S.

Figure 5. ^1H NMR spectrum of 6-IMCA320S.

Next, we used the fully converted, bromide-free imidazolyl derivative for further polyelectrolyte preparation. We wished to investigate whether the remaining basic nitrogen of the 6-IMCA imidazole substituents could be exploited to provide access to bromide-free polyelectrolyte. As Scheme 3 shows, methyl iodide (10 equiv/AGU) was employed as electrophile to react with 6-IMCA in NMP at 50 °C. ^1H NMR spectroscopy (Figure S7)

confirms successful formation of the targeted cationic ionomer, 6-MeIMCA. Nucleophilic attack of the remaining basic imidazole 3-nitrogen upon methyl iodide creates a positive charge at the imidazolium atom. The resonances at 8-10 ppm are due to the aromatic protons of the imidazolium substituent, while the resonance around 3.8 ppm indicates imidazolium N-methyl, confirming successful conversion to the targeted cationic derivative. DS(N-methyl) calculated by ^1H NMR is 0.39. ^{13}C NMR spectroscopy (Figure S8) also shows a new resonance at 37 ppm which we assign to the methyl group appended to the cationic nitrogen atom of imidazole. Based on ^1H NMR and FTIR spectroscopy (Figure S9), it appears that the quaternization reaction does not greatly affect the acetyl groups of CA320S. DS(Ac) 1.70 calculated by ^1H NMR (supported by the strong carbonyl absorbance at ca. 1750 cm^{-1} in the FTIR spectrum) means that there is no significant loss of ester groups of cellulose esters during imidazole displacement and quaternization by methyl iodide.

Scheme 3. Quaternization of 6-IMCA320S with methyl iodide.

3.6. Water solubility and zeta potential of 6-PyrCA320S and 6-MeIMCA320S

Having successfully prepared cationic cellulose-based polyelectrolytes from a commercial cellulose ester (CA320S), an important question was whether these amphiphilic polyelectrolytes would have sufficient water solubility for the anticipated biomedical applications. We found that relatively concentrated (50 mg/mL) aqueous solutions could be

prepared from either 6-PyrCA320S or 6-MeIMCA320S, in addition to their good organic solubility in DMF and DMSO. It is interesting to note that 6-PyrCA320S and 6-MeIMCA320S are more water soluble than pyridine ($DS(\text{pyridinio}) = 0.71$) or 1-methylimidazole ($DS(\text{methylimidazolio}) = 0.79$) substituted cellulose acetate (acetylated at the C-2/3 positions) derived from MCC (Liu, Liu, Esker & Edgar, 2016), in spite of the overall lower charge density of the CA 320S-based polymers. Improved solubility could be due in part to the reduced stereoregularity of the CA 320S derivatives (leading to less tendency to crystallize or self-associate), and could also be influenced by the fact that CA320S retains some hydrophilic hydroxyl groups at C-2 and C-3.

Since the cationic polyelectrolytes are very soluble in water, we measured zeta (ζ)-potentials for aqueous solutions (0.1 mg/mL) of our polyelectrolytes to confirm the expected charge. Zeta potential measurement is a simple but powerful tool to prove the successful preparation of cationic cellulose ionomers by phosphine-catalyzed bromination and subsequent aromatic amine displacement, and to affirm polyelectrolyte nature prior to testing in biomedical and other potential uses. The zeta-potential values for 6-PyrCA 320S and 6-MeIMCA320S solutions were $+ 52.43 \pm 1.16$ mV and $+ 52.68 \pm 0.19$ mV, respectively, consistent with the proposed polyelectrolyte structures.

4. Conclusions

A simple and efficient (high yields and conversions) strategy has been developed for preparing renewable-based copolymers containing useful leaving groups at C-6 by PPh_3/NBS bromination of commercial cellulose esters, with complete regio- and chemoselectivity. We show that displacement of these new C-6 bromides to afford uncharged

products (6-azide, 6-imidazolyl) is quantitative, providing derivatives with high promise for introduction of amine or amide (azide) or cationic/zwitterionic (6-imidazolyl) functional groups to form derivatives that contain no residual 6-bromide and will therefore be devoid of toxicity therefrom. The 6-bromo-6-deoxy copolymers are also useful precursors to cationic polyelectrolytes, demonstrated for 6-BrCA320S by nucleophilic displacement with aromatic amines including pyridine and 1-methylimidazole. These polyelectrolytes exhibit very good solubility in water. Note that it should be possible to eliminate the (for some purposes) undesired residual 6-Br substituents by polishing reaction with a neutral nucleophile, such as imidazole or a thiol. Moreover, we demonstrated that neutral 6-IMCA320S, prepared by imidazole displacement reaction of 6-BrCA320S in quantitative conversion and high yield, provides an efficient pathway to bromide-free polyelectrolytes, by quaternization of the other imidazole nitrogen with methyl iodide.

It is perhaps surprising and certainly satisfying that such halogenation and displacement reactions can be carried out in the presence of somewhat labile groups like acetate esters. Further, it is gratifying that these methods provide access to what promises to be a wide variety of novel cationic and zwitterionic polysaccharide ester derivatives, in a few steps from readily available commercial polymers. Availability of these polysaccharide-based electrolytes will accelerate structure-property relationship studies for a variety of emerging uses including complexation of poly(nucleic acids) for delivery to cell nuclei, delivery of anionic drugs, and epithelial tight junction opening for oral protein delivery. The ability to prepare neutral 6-IMCA320S by related, quantitative displacement reactions of 6-BrCA320S provides access to other interesting applications requiring zwitterionic polyelectrolytes (e.g. antifouling applications), by reaction with propane sultone or related

reagents. The simple methodology for preparing water-soluble (and amphiphilic) derivatives from relatively hydrophobic cellulose esters is especially notable, and valuable. In order to apply these cationic polyelectrolytes to biomedical and pharmaceutical application areas, it will be of particular interest for us to investigate the interactions between the cationic cellulose derivatives and biomolecules such as nucleic acids or certain anionic proteins, as well as their cellular toxicity and antimicrobial activity.

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