Regioselective Chlorination of Cellulose Esters

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Abstract

Chemical modification of cellulose has been of growing interest, owing to the abundance and processing challenges of natural cellulose. To date, etherification and esterification are the most effective strategies to modify physicochemical properties of cellulose and append new functionalities. However, they typically require relatively harsh conditions, thus limiting introduction of new functional groups. An alternative strategy to synthesize novel cellulose derivatives is to append a good leaving group to cellulose backbone, followed by nucleophilic substitution reaction. Though tosylation and bromination of cellulose are frequently used, they have drawbacks such as chemo- and regioselectivity issues, high cost, and difficulty in purification.

We have successfully developed a method to chemo- and regioselectively chlorinate cellulose esters using MsCl. Compared to bromination of cellulose typically used, this chlorination method has many advantages, including low cost of reagents and ease of separation. The chlorinated cellulose esters are useful intermediates for appending new functionalities by displacement reactions. We have synthesized a library of cellulose ester derivatives by this chlorination/nucleophilic substitution strategy, including cationic and anionic cellulose ester derivatives. These cellulose ester derivatives possess great potential

for various applications, including amorphous solid dispersion, tight junction opening, anionic drug delivery, and gas separation membranes.

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General Abstract

Cellulose is one of the most abundant natural materials on earth, making up from 30-40% of the weight of every plant on Earth. However, natural cellulose is hard to process into objects for our use, because it can't be dissolved in water or other simple solvents, and also can't be melted (it decomposes before it melts). Chemical modification is a useful method to tailor cellulose properties. Conventional methods to do that (making esters and ethers) are limited in scope by harsh reaction conditions.

This thesis focuses on investigating a novel strategy to modify cellulose esters and prepare novel cellulose ester derivatives for various applications. Compared to other methods, it shows great potential in industrial applications, because of the low cost of reagents, high efficiency and selectivity, and ease of processing the products. By employing this method, we have prepared different cellulose ester derivatives, with a wide range of future applications, such as in membranes for purifying gases, and for effective drug delivery. Besides cellulose esters, the new method is likely to be suitable for modification of many other natural polysaccharides and their derivatives.

Dedication

To my advisor, Prof. Kevin J. Edgar

Acknowledgments

I would like to give my greatest appreciation to my research advisor, Prof. Kevin J. Edgar. I have gained more scientific insights and personal confidence inspired by his guidance, passion, and knowledge. He is the best advisor and mentor to work with and is also a great person I can learn from in my life.

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Attribution

Dr. Shu Liu has contributed to the chlorination of CA320S and subsequent displacement reaction by diethylamine in Chapter 3 and offered valuable suggestions in this work. He was a co-author in this paper.

Table of Contents

Outline

Cellulose is one of the most abundant natural materials, and acts as the primary structural reinforcement polymer in plants. Applications of cellulose are impeded by insolubility in common solvents and lack of thermoplasticity. Chemical modification of cellulose is an efficient method to modify its physicochemical properties and append new functionalities. Conventional modification strategies, including etherification and esterification, typically depend on using a strong acid or base, constraining the introduction of sensitive functional groups. Increasing demands for renewable biomaterials require more efficient and powerful methods to modify cellulose and its derivatives.

A useful strategy to synthesize novel cellulose derivatives is to append a good leaving group to cellulose backbone, followed by nucleophilic substitution reactions. Although tosylation and bromination of cellulose are frequently used in cellulose chemistry, they have drawbacks including chemo- and regioselectivity issues, high cost of reagents, and difficulty in purification and separation. Chloride is a frequently used leaving group in organic chemistry. However, there are few reports on chlorination of cellulose and its derivatives, while regioselective chlorination of many other polysaccharides, like amylose, pullulan, and inulin have been accomplished by reaction with methanesulfonyl chloride. My master's research in this thesis presents an efficient method for regioselective

chlorination of cellulose esters by methanesulfonyl chloride. We further demonstrate herein the ability to further displace C6-Cl by different nucleophiles, affording a wide variety of cellulose ester derivatives.

An outline of this thesis is as follows: Chapter 2 gives an introduction to polysaccharides, including cellulose, curdlan, chitin, chitosan, and glycosaminoglycans (GAGs). A detailed discussion of chemical and chemoenzymatic sulfation of polysaccharides is also presented. Chapter 3 describes a chemo- and regioselective method to chlorinate cellulose esters by using methanesulfonyl chloride. The resulting chlorinated cellulose esters prepared are useful intermediates for appending new functional groups by nucleophilic displacement reactions. Employing this strategy, various cellulose ester derivatives, including cationic and anionic polyelectrolytes, are synthesized. Chapter 4 provides a summary of the research and outlines our future plans for utilizing our chlorination method, and other techniques, to synthesize GAG analogues, including selectively sulfated GAGs.

Chapter 1: Review of Literature: Sulfation of Polysaccharides

1.1 Introduction to polysaccharides

Polysaccharides are biopolymers composed of repeating monosaccharides linked by glycosidic bonds, and they have great structural and functional diversity. Their structural diversity comes from differences in monosaccharide structure, linkage, stereochemistry, and branching. Polysaccharides are highly abundant in nature and can be obtained from many renewable sources. For example, cellulose and starch can be found in plants, while chitin and heparin can be extracted from animals. Natural polysaccharides play important biological roles, including structure reinforcement, energy storage, cellular communication, and signal identification. Due to their non-toxicity, renewability and biocompatibility, polysaccharides have great industrial applications in textiles, food, pharmaceuticals, biofuels, and environmental protection.

1.1.1 Cellulose

First discovered by French chemist Anselme Payen in 1838, cellulose is among the most abundant polymeric raw materials with interesting properties and applications. It acts as the primary structural polymer in trees and other plants and as a storage polymer for photosynthetic products.[1] It can be obtained from wood, cotton fibers, bacteria, and certain animals (e.g. tunicates). Due to its vast abundance, low cost, biocompatibility, and

biodegradability, cellulose has been the most commercially important polysaccharide with various applications.

Cellulose is a linear homopolysaccharide consisting of 1→4 linked β-D-glucopyranose monosaccharides (Figure 1.1). Each anhydroglucose unit (AGU) has three hydroxyl groups, including one primary hydroxyl group at the C6 position and two secondary hydroxyl groups at the C2 and C3 positions. These hydroxyl groups result in an extensive hydrogen bonding network, which leads to the semi-crystalline morphology of cellulose.[2] The degree of polymerization (DP) of cellulose varies with the origin and treatment of the raw material. For instance, wood pulps have DP values in a range of 300 to 1700, while DP of cotton fibers and bacterial cellulose can vary from 800 to 10,000.

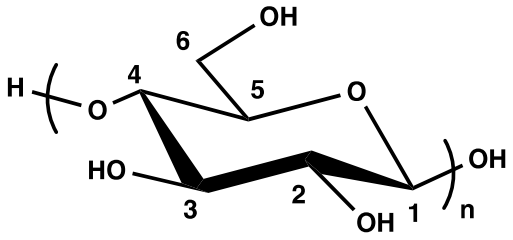


Figure 1.1 Structure of cellulose

Cellulose has desirable properties like low elasticity, biodegradability, thermal stability, and low toxicity. It has been a useful material for paper, cellulose regenerated fibers, films, coating and packing.[3] However, wider applications are limited by its insolubility in water and common organic solvents. Furthermore, cellulose decomposes before melting and therefore it is not melt-processable. To overcome these processing issues, cellulose is derivatized to create processable derivatives with enhanced properties.

Etherification and esterification are the most effective industrial strategies to introduce desired properties to cellulose derivatives.[4] Cellulose ethers and esters have been widely used in many areas including coatings, optical films, drug delivery, membranes, and composites.[5] Besides etherification and esterification, there has been a growing number of modern chemistry methods to prepare novel cellulose derivatives in the past 20 years, such as bromination/tosylation/chlorination followed by nucleophilic substitution reactions[6,7] and olefin cross metathesis.[8,9]

1.1.2 Curdlan

Curdlan is a bacterial polysaccharide that has aroused great interest in the past few decades due to its unique rheological properties and promising biological activities. It was first discovered in 1966 by Harada and coworkers[10] and the structure of curdlan was elucidated as early as 1968[11]. Curdlan consists of D-glucopyranose monosaccharides linked by β-1→3 linkages, without any branching (Figure 1.2). It is an extracellular capsular polysaccharide with DP up to 12,000, and can be produced by non-pathogenic bacteria such as *Agrobacterium* biovar.[12]

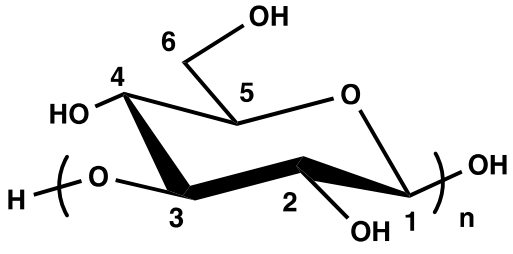


Figure 1.2 Structure of curdlan

Curdlan shows enhanced solubility versus other linear polysaccharides, such as cellulose. Curdlan is soluble in dilute alkali (0.25 M NaOH), formic acid, and some polar aprotic solvents including dimethyl sulfoxide (DMSO), *N*-methylmorpholine *N*-oxide (NMMO), and *N,N*-dimethylacetamide (DMAc) containing lithium chloride (LiCl), while like many common polysaccharides, curdlan is insoluble in water and most other organic solvents. Curdlan exhibits unique gelation properties. Heating curdlan aqueous suspension will generate two types of gels, depending on the maximum temperature used. A high-set, thermally irreversible gel can be formed when a curdlan aqueous suspension is heated to 80 °C, and a low-set, thermally reversible gel can be obtained by heating the suspension to 55 °C.[13] Gelation has been attributed to curdlan's helical structure change at the elevated temperatures. At room temperature, curdlan has a mixture of single helix and loose triple helix structure, while at higher temperatures curdlan has a more condensed triple helix structure.

Curdlan has been used widely in the food industry and in pharmaceutics due to its gelation property, low toxicity, and biocompatibility. It was approved as a food additive in the United States in 1996 by the Food and Drug Administration (FDA). It is frequently used as a thickening agent or fat-mimic substitute in the food industry. Curdlan gel has been demonstrated to be effective as a sustained release drug delivery system. Curdlan and its derivatives display a variety of biological activities themselves, including antitumor, anti-infective, anti-inflammatory, and anticoagulant activities.[14]

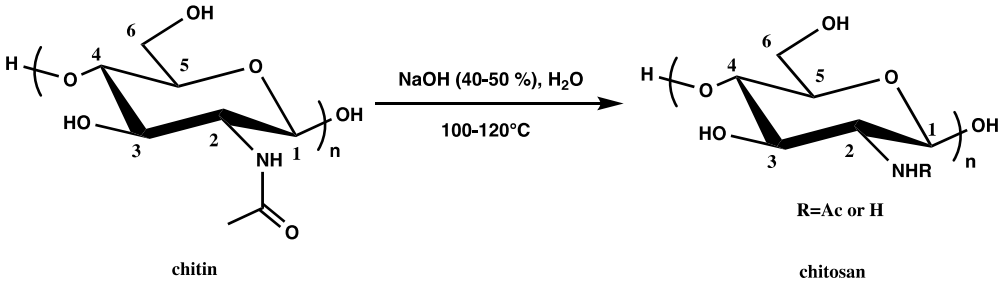
1.1.3 Chitosan and chitin

Chitin, a highly abundant amino polysaccharide, is composed of 2-acetamido-2-deoxy-β-D-glucose linked by β (1→4) glycosidic bond. Chitin is similar to cellulose in many aspects including chemical structure, biological functions, and physicochemical properties. It serves as a structural component in many organisms, reinforcing natural matrix composites. Chitin can be extracted from many sources, including molluscs, crustaceans, insects, fungi, and algae. Chitin, as a sustainable, biocompatible, and biodegradable material, shows great potential including as a chelating agent[15], drug carrier[16], and in membranes[17]. However, its vast potential for useful applications is impeded by poor solubility and lack of thermal processability. Chitin is insoluble in common organic solvents and water, due to its extensive semicrystalline structure caused by hydrogen bonding, and it decomposes before melting.[18]

Chitosan, prepared by deacetylation of chitin, is a random copolymer of 2-amino-2-deoxy-β-D-glucose and 2-acetamido-2-deoxy-β-D-glucose, linked by β (1→4) linkages (Scheme 1.1). The degree of deacylation in chitosan varies from 40 % to 98 %, depending on starting material chitin and the deacylation methods. Chitosan is soluble in dilute acidic solutions below pH 6.0, as a result of the strong basicity of the free amine groups (pKa 6.3). At low pH, the amine groups are protonated and become positively charged, making chitosan a water-soluble cationic polyelectrolyte. The most commonly used solvent system

for dissolving chitosan is 1% acetic acid at pH around 4.0.[19] The significant improvement in water solubility versus chitin enhances the processability of chitosan, making film formation, coating, precipitation, and freeze-drying feasible.

Chitin and chitosan, owing to their sustainability, biocompatibility, non-toxicity, and biodegradability (even *in vivo*), have many applications in water purification, wound healing, production of paper, packing, and cosmetics.[20] Furthermore, chitin and chitosan can be readily processed into gels[21], membranes[22], fibers[23], nanoparticles[24], and sponges[25], enhancing their biomedical applications in antibacterial materials, tissue engineering, drug delivery, and gene delivery.



Scheme 1.1 Synthesis of chitosan by alkaline hydrolysis of chitin

1.1.4 Glycosaminoglycan (GAG)

Glycosaminoglycans (GAGs), one of the most important macromolecule families that affect cellular functions, are a large family of long-chain, unbranched, biologically active polysaccharides, based on disaccharide repeat units. The disaccharide repeat unit usually contains a uronic acid (glucuronic acid or iduronic acid) or galactose (in the case of keratan sulfate), along with an amino sugar (glucosamine, *N*-acetylglucosamine or *N*-

acetylgalactosamine).[26] GAGs include heparin (HP), heparan sulfate (HS), dermatan sulfate (DS), chondroitin sulfate (CS), keratan sulfate (KS) and hyaluronic acid (HA) (Figure 1.3). Except for hyaluronic acid, GAGs are usually sulfated to various degrees and in different patterns.[27] Sulfate groups are crucial to GAG natural function because recognition and modulation of most proteins are commonly induced by the *O*-sulfate or *N*-sulfate groups on the GAG chains. GAGs exist covalently linked to proteins as part of proteoglycans, except for HA, which has high non-covalent affinity for certain proteins. Most GAGs are negatively charged, due to carboxyl and sulfate groups on the polymer. GAGs play important roles in many essential biological processes, including blood coagulation, growth, cancer, inflammatory response, immune response, and pathogen infection.[28–30]

1.2 Chemical sulfation of polysaccharides

Polysaccharide sulfates are polysaccharides bearing sulfuric acid half ester moieties. They are widely distributed in nature with a variety of important biological activities and biomedical applications. The most important natural polysaccharide sulfates are GAGs extracted from animals, which play key roles in physiological and pathological processes. Inspired by the biological importance of natural polysaccharide sulfates, researchers have employed chemical sulfation of natural unsulfated polysaccharides, such as cellulose, curdlan, and chitosan, to prepare novel polysaccharide sulfates. Because the half ester

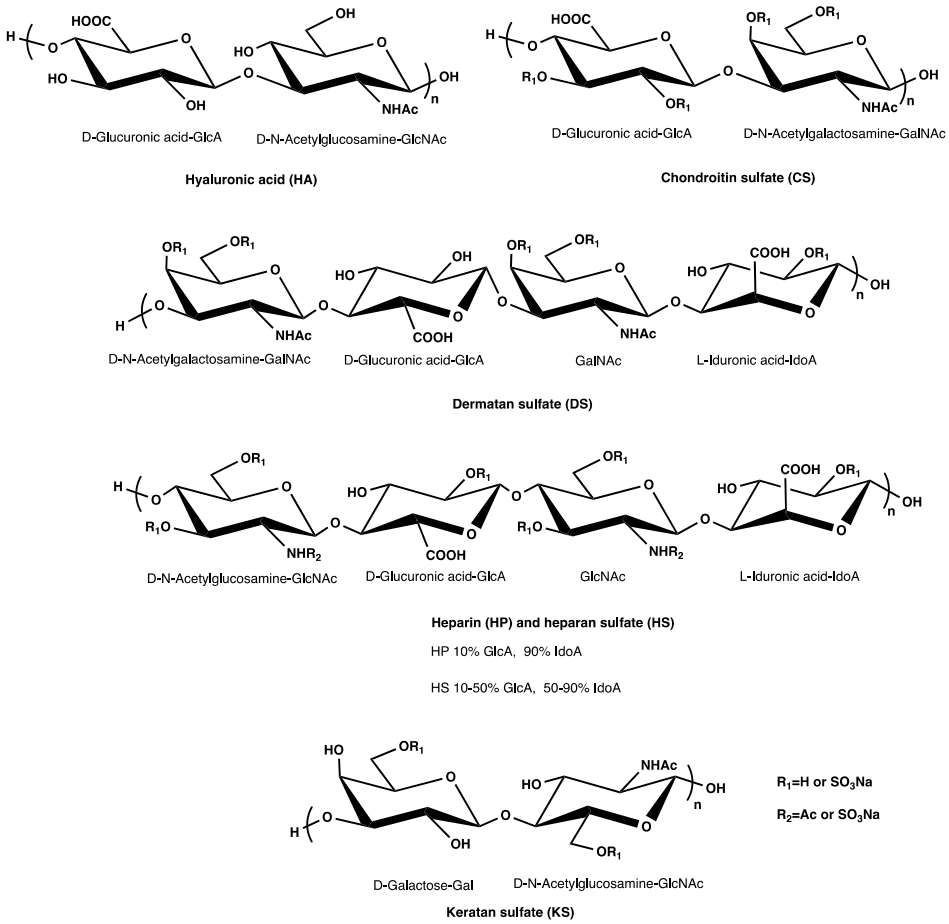


Figure 1.3 Structures of HA, CS, DS, KS, HP, and HS

moiety in its protonated form (-OSO_3H) is so acidic that it can lead to chain degradation, semi-synthetic polysaccharide sulfates often are isolated in salt form. Similar to natural polysaccharide sulfates, semi-synthetic polysaccharide sulfates also exhibit a large number of biological activities.[31] Furthermore, polysaccharides can be sulfated chemically at large scale, affording products with defined structural features and purity, and at low cost.

Traditional sulfation reagents like sulfuric acid, sulfuryl chloride, and other reagents including1-piperidinesulfonic acid, chlorosulfonic acid, and sulfamic acid were first employed for sulfating polysaccharides. However, significant depolymerization was

always observed, due to the breakage of glycosidic bonds under strong acidic conditions.[32] Moreover, these sulfation reagents are hazardous and should be handled carefully. Sulfur trioxide complexes with amides (DMF) or amines (including pyridine and trimethylamine) in polar aprotic solvents provide an alternative method to sulfate polysaccharides, because of the mild reaction conditions, stability and mild acidity of the sulfation reagents, and ease of handling of those reagents. In several cases, depolymerization and cleavage of acid labile functional groups was still observed. It has been reported that adding an acid scavenger, e.g. 2-methyl-2-butene, can minimize side reactions caused by acidic conditions to the greatest extent.[33] All these sulfation reagents show preference for reaction with primary hydroxyl groups, but none of these sulfation reagents are entirely regioselective. With increased understanding of the importance of sulfate distribution patterns in the biological activities of polysaccharide sulfates, regioselective sulfation of polysaccharides is becoming a more important goal. Protecting group strategies have proven useful for regioselective synthesis of polysaccharide derivatives. Combined with sulfation reactions, they allow more regioselective sulfation of polysaccharides. A detailed discussion of sulfation of cellulose, curdlan, chitin, and chitosan is presented here.

1.2.1 Sulfation of cellulose

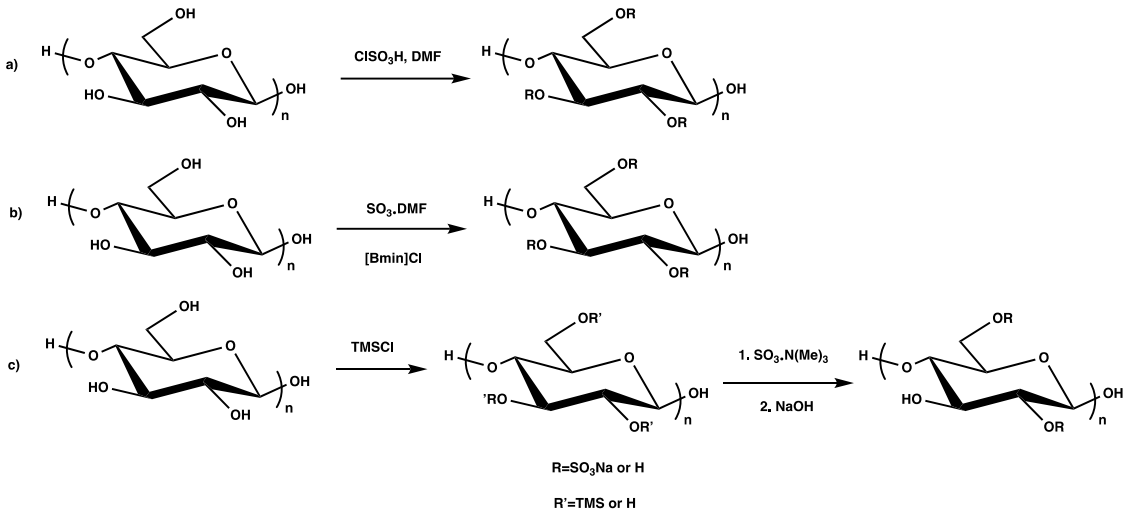
Cellulose sulfates have been of growing interest in the past few decades, due to their potential applications in films[34], anionic polyelectrolytes[35,36], and their biological activities,

such as anticoagulant[37], antiviral[38], and anti-human immunodeficiency virus (HIV) activities[39]. Owing to the abundance of cellulose, its sulfation has been studied most extensively among polysaccharides. Cellulose sulfates can be obtained under both heterogeneous and homogeneous conditions. Due to the limited solubility of cellulose, heterogeneous sulfation is a straightforward way to synthesize cellulose sulfates. For example, cellulose can be sulfated heterogeneously with H_2SO_4 in ethanol or *n*-propanol.[40,41] This reaction is usually performed below 0 °C to minimize acid-catalyzed chain cleavage. To minimize chain degradation, an alternative method utilizes SO_3•pyridine or SO_3•DMF as the sulfation reagent, H_2SO_4 as the catalyst, and dimethylformamide (DMF) as the solvent.[42] Like many other heterogeneous reactions on cellulose, the problem for heterogeneous sulfation is non-uniform sulfate group distribution. The amorphous part of the cellulose can be sulfated while the crystalline part remains unreacted. Cellulose can also be homogeneously sulfated in a variety of solvent systems that can be used to dissolve cellulose, such as N_2O_4/DMF[43], DMAc/LiCl[44], and ionic liquids[45–47]. Compared to heterogeneous sulfation, homogeneous sulfation usually can be carried out under milder reaction conditions. As a result, usually less chain degradation is observed. Furthermore, under homogeneous conditions, the cellulose sulfates obtained are presumed to have even sulfate distributions along and between chains, and exhibit good solubility. Both heterogeneous and homogeneous sulfation show similar regioselectivity: C6 > C2 > C3.[4] Sulfation at C6 is always preferred, as a result of the wider

approach angles of the primary hydroxyl groups. Hydroxyl groups at C2 are found to be more reactive than at C3 positions for sulfation. In some cases, cellulose-6-sulfate ($DS_{sulfate}$ = 0.55)[48] and cellulose-2, 6-disulfate ($DS_{sulfate}$ = 2.0)[49] can be synthesized with almost complete regioselectivity by careful choice of sulfation reagents, solvents, and reaction conditions.

Cellulose derivatives can also be used as substrates for sulfation reactions, benefitting from their improved organic solubility versus cellulose. Such strategies are known as quasi-homogeneous sulfation. Cellulose acetate, cellulose nitrate, and trimethylsilyl cellulose are frequently used in cellulose sulfate synthesis. Cellulose acetate or cellulose acetate generated *in situ* have been studied extensively in the synthesis of cellulose sulfates. This process usually involves two steps: acetosulfation and subsequent deacylation at room temperature to avoid possible desulfation reactions.[32] In 1995, Philipp et al. reported synthesis of cellulose sulfates using $ClSO_3H/Ac_2O$/DMF at 50° C, followed by hydrolysis of ester groups under alkaline conditions.[48] In 2011, Zhang et al. employed this method to synthesize cellulose sulfates with degree of substitution of sulfate groups ($DS_{sulfate}$) ranging from 0.21 to 1.71, varying the reaction temperature, sulfation reagents, acylation reagent, and solvents.[50] In many cases, this method shows similar regioselectivity trends as described above (C6 > C2 >> C3). Cellulose silyl ethers are also useful intermediates for sulfating cellulose. For example, it was reported that trimethylsilyl cellulose (DS_{TMS} = 1.55) can be sulfated with $ClSO_3H$ in DMF, followed by treatment with NaOH in ethanol to

cleave the residual trimethylsilyl groups (residual trimethylsilyl groups come from TMS groups that are not hydrolyzed under acidic conditions or substituted by sulfation reagents).[51] It is worth noting that an almost completely regioselectively substituted cellulose 6-*O*-sulfate was obtained, with $DS_{sulfate}$ at the C6 0.95 (determined by ^{13}C NMR). In 2003, Richter and Klemm reported sulfation of nearly fully substituted trimethylsilyl cellulose (DS_{TMS}=2.9) with different SO_3 complexes.[52] When using SO_3•DMF, sulfation occurred preferentially at C6. The $DS_{sulfate}$ at the C6 position can be as high as 0.95 while $DS_{sulfate}$ at C2 is about 0.51. When SO_3•triethylamine was employed as the sulfation reagent, a preference for sulfation at the C2 position was observed. ^{13}C NMR analysis revealed that the $DS_{sulfate}$ at C2 was 0.42 and $DS_{sulfate}$ at C6 position was 0.21. The result revealed that both the total degree of sulfation and regioselectivity depend on the sulfation reagents used. Sulfation of cellulose silyl ethers occurs at C2 and C6 and sulfation at C3 is not detected by ^{13}C NMR. Thus, this approach could be employed to synthesize cellulose-2,6-*O*-disulfate derivatives. Cellulose nitrate, typically generated *in situ* by treating cellulose with N_2O_4/DMF, can be sulfated by various sulfation reagents.[43,48] The regioselectivity of sulfation of cellulose nitrate is similar to sulfation of cellulose silyl ether derivatives. Only sulfation at C6 and C2 is observed, and the preference of C6 and C2 is dependent on sulfation reagents and reaction temperature. Investigation has shown that when using SO_3 as the sulfation reagent, sulfation at C2 dominates at low temperature (-20° C), while sulfation at C6 is preferred at room temperature (20° C).[43]



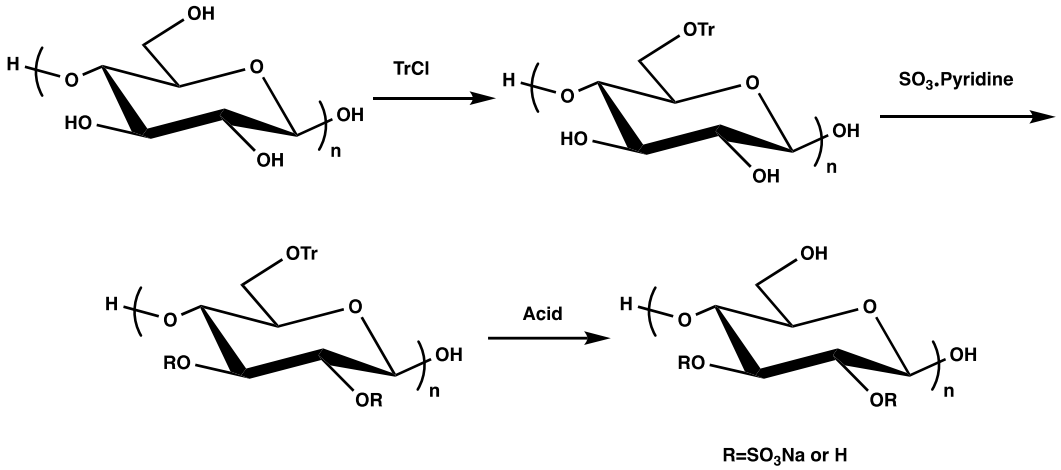
Scheme 1.2 Examples of sulfating cellulose: a) heterogeneous sulfation, b) homogeneous sulfation, c) quasi-homogeneous sulfation

Both cellulose sulfates and many other polysaccharide sulfates synthesized are typically analyzed by FT-IR, ^{1}H NMR, ^{13}C NMR, and elemental analysis. To accurately determine the position of substitution is very challenging. Most of the publications on sulfation of polysaccharides use quantitative ^{13}C NMR spectra as the only evidence to determine $DS_{sulfate}$ at every position, despite the inaccuracy and imprecise nature of quantitative ^{13}C NMR spectra and possible errors generated in peak integration due to peak overlaps. The Mischnick group has developed a method to determine the substitution pattern in cellulose sulfates quantitatively.[53,54] Cellulose sulfates are initially permethylated by methyl iodide (MeI) in DMSO, followed by replacement of sulfate groups by acetate groups (Ac_2O) at high reaction tempearature for a long time (14 h) (in order to complete desulfation and acetylation), and replacement of acetate group by deuteromethyl ethers ($NaOH/CD_3I$). Finally, the permethylated cellulose samples are hydrolyzed to

monosaccharides (enzymatic or chemical approach). In the monosaccharides, the *O*-methyl-d_3 groups represent the positions of the original sulfate groups, while the *O*-methyl groups represent the positions of the unreacted hydroxyl groups. The monomer compositions were quantitatively determined by GC-MS. This method is extremely useful in assigning the substitution position. However, it is surprising that this method has only been applied in a few cases. A simple and accurate method to measure the sulfate distribution would be promising and facilitate the synthesis of both cellulose sulfates and many other polysaccharide sulfates with complete regioselectivity.

Regioselective synthesis of cellulose sulfates is challenging, due to the low reactivity and the small reactivity differences between cellulosic hydroxyl groups. Until now, the most effective strategy in the regioselective synthesis of cellulose sulfates is employing protecting groups. Triphenylmethyl group (trityl group), 4-methyloxy-triphenylmethyl group (MMTr), and 4,4'-dimethoxy-triphenylmethyl group (DMTr) have been used widely to protect primary hydroxyl groups of polysaccharides. Owing to its steric demands, triphenylchloromethane will react preferentially with primary hydroxyl groups and the secondary hydroxyl groups remain largely unreacted. Moreover, the trityl or methoxytrityl group can be easily removed under acidic conditions at room temperature. In 2000, Heinze and co-workers reported regioselective synthesis of cellulose 2,3-disulfate through three step reactions: first, introduction of trityl groups to cellulose at C6, then sulfation of secondary hydroxyl groups at C2 and C3 using SO_3• pyridine, and finally removal of trityl

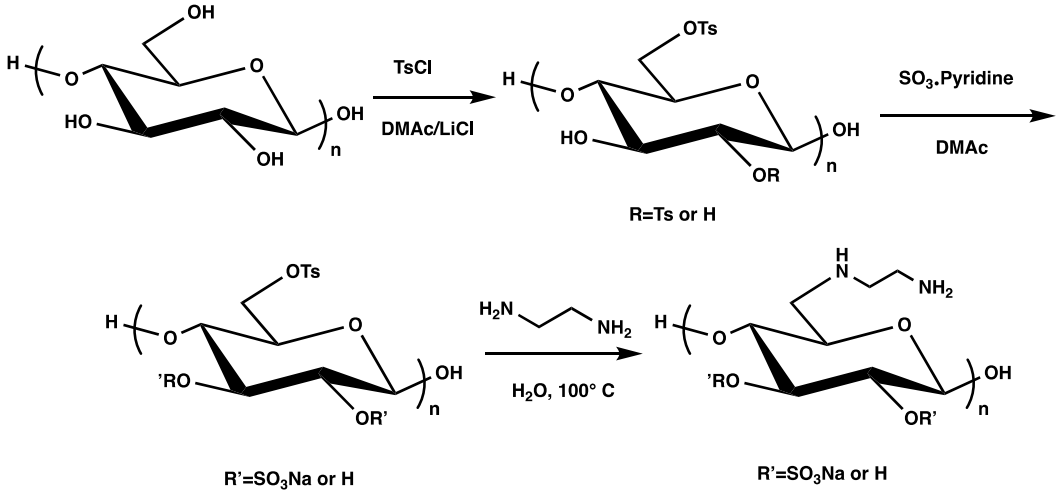
groups in aqueous acid (Scheme 1.3).[44] Similar strategies have also been applied to regioselective synthesis of many other polysaccharide sulfates. By a similar method, a glucogalactomannan from *Artemisia sphaerocephala*[55], guar gum[56], and unsulfated porphyran[57] have been regioselectively sulfated to yield the corresponding polysaccharide sulfates with sulfate groups distributed only at former secondary hydroxyl positions.



Scheme 1.3 Regioselective synthesis of cellulose-2,3-disulfate

Synthesis of cellulose sulfates bearing other substituents, such as methylated cellulose sulfate[53], carboxyl cellulose sulfate[58], oxyethylated cellulose sulfate[59], and amino cellulose sulfate[60], has been further explored. For instance, in 2012 Heinze and co-workers reported regioselective synthesis of amino cellulose sulfates as polyzwitterions (Scheme 1.4).[60] Cellulose in DMAc/LiCl was reacted with *p*-toluenesulfonyl chloride (tosyl chloride) in the presence of a base. The tosyl group was mainly introduced to the C6 position due to lower steric hindrance there, despite some tosylation at C2 and C3. Then tosyl cellulose was sulfated with SO_3•pyridine at the C2 and C3 position in DMAc, affording a water-

soluble tosyl cellulose sulfate. Subsequent treatment with NaOH aqueous solution transformed sulfonic acid half esters into sodium salts. In the final S_N2 reaction, tosyl groups were replaced by the nucleophile, 1,2-diaminoethane, to yield amino cellulose sulfates. The water solubility of the amino cellulose sulfates depended on the pH value due to the existence of both sulfate and amine groups. Amino cellulose sulfate (DS_{amine}=0.32, $DS_{sulfate}$=1.21) is completely water-soluble at pH 11.5. At pH around 9, colloids were observed and a precipitate formed at pH < 9. It was proposed that when pH > 11.5 all amine groups are deprotonated and the polymer is soluble because of the sulfate groups. The precipitation formed at pH < 9 was proposed to be caused by protonation of amine groups and formation of an insoluble zwitterionic polymer, with the decrease of pH.



Scheme 1.4 Synthesis of amino cellulose sulfate

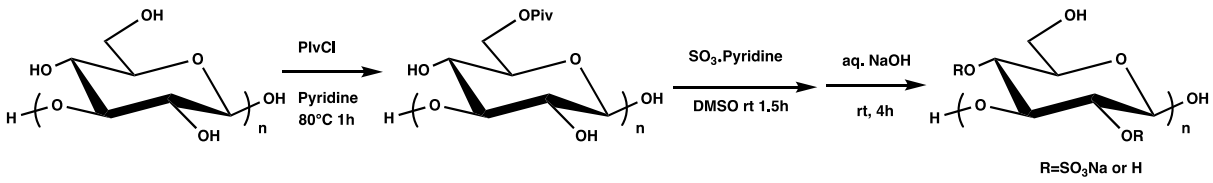
1.2.2 Sulfation of curdlan

Curdlan sulfates have been of great interest, owing to their high anti-HIV activity. In

1990, Yoshida and coworkers first reported homogenous sulfation of curdlan by piperidine-*N*-sulfonic acid (PSA) in DMSO.[61] Curdlan sulfates with $DS_{sulfate}$ from 0.35 to 1.6 were synthesized by varying the reaction time, temperature, and molar ratios of sulfation reagents. Regioselectivity studies revealed that the relative hydroxyl group reactivity was O-6 > O-2 >> O-4, as revealed by ^{1}H, ^{13}C, and relayed-COSY NMR spectra. Sulfation occurred mostly at C6 at lower DS, and then at C2 as well with increasing DS. Even at $DS_{sulfate}$ as high as 1.6, very few sulfate groups at C4 were observed. In 1993, Osawa et al. studied the impact of sulfation conditions on anti-HIV activity by treating curdlan with SO_3• pyridine complex in DMF under homogeneous or heterogeneous conditions.[62] Analysis of the curdlan sulfates synthesized by homogeneous sulfation indicated that the regioselectivity was C6 > C2 > C4. However, under heterogeneous conditions, there was little difference between the reactivity of the two secondary hydroxyl groups O-2 and O-4, while sulfation of the primary hydroxyl groups O-6 was preferred. Curdlan sulfates obtained by heterogeneous sulfation exhibited unwanted cytotoxicity, while curdlan sulfates prepared by homogeneous sulfation displayed low cytotoxicity, illustrating the importance of sulfation reagents and sulfation conditions used.

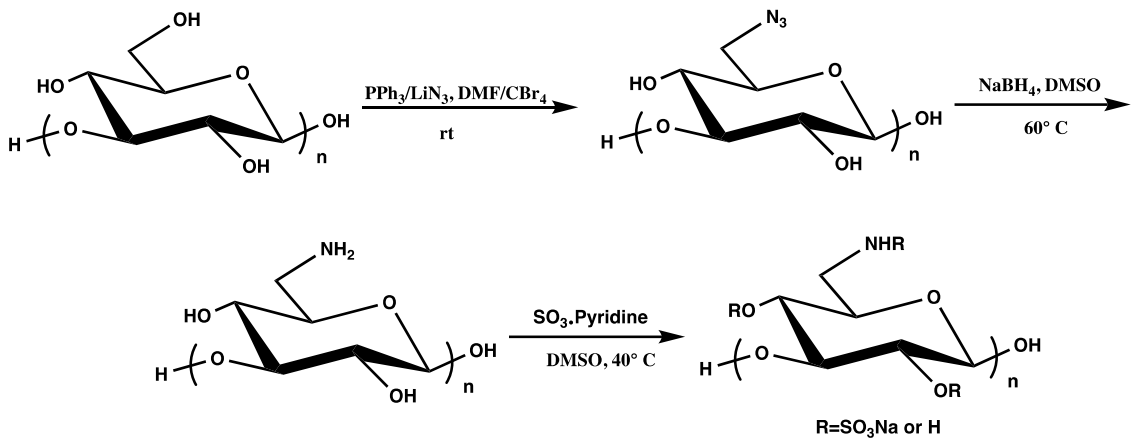
Regioselective sulfation of curdlan has also been achieved by protecting group strategies. In 1997, Gao et al. reported regioselective synthesis of curdlan-2,4-disulfate using pivaloyl (trimethylacetyl) groups to selectively protect the primary hydroxyl groups (O-6) (Scheme 1.5).[63] Pivaloyl groups are extremely sterically demanding, and thus

introduction to the less hindered primary hydroxyl groups at C6 is favored. Curdlan was first treated with pivaloyl chloride in pyridine at 80° C to protect the primary hydroxyl groups. Then the protected curdlan was sulfated by SO_3• pyridine in DMSO, followed by alkaline hydrolysis of the pivaloyl groups. The curdlan sulfate obtained had a $DS_{sulfate}$ = 0.39 at C2 and a $DS_{sulfate}$ = 1.00 at C4, while no sulfation at C6 was detected, as measured by ^{13}C NMR spectra. The regioselectively synthesized curdlan sulfates exhibited similar anti-HIV activity and the authors concluded that the anti-HIV activities of curdlan sulfates depended upon DS rather than upon sulfation positions.



Scheme 1.5 Synthesis of curdlan 2,4-disulfate

In order to further explore the relationship between structure and anti-HIV activity, sulfation of curdlan derivatives has also been reported. For example, Borjihan et al. reported the sulfation of aminated curdlan in 2003 (Scheme 1.6).[64] 6-Amino-6-deoxy-curdlan was prepared by one-pot bromination and azide displacement, followed by azide reduction. Subsequent sulfation of the amino and hydroxyl groups was carried out by SO_3• pyridine in DMSO at 40° C. Sulfated amino curdlan showed high anti-HIV activity and low cytotoxicity.

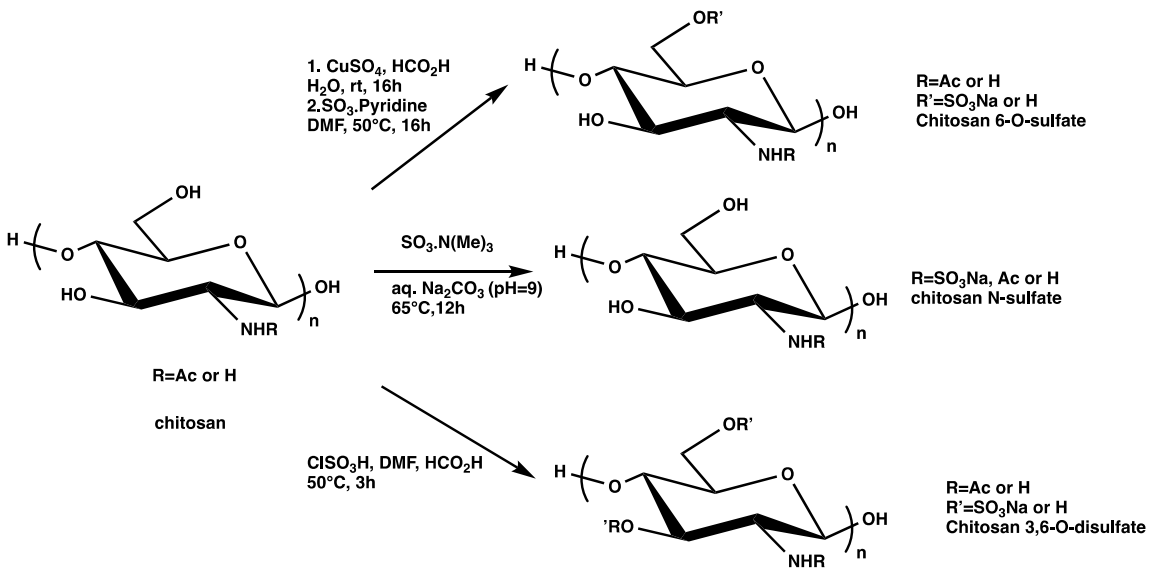


Scheme 1.6 Sulfation of 6-amino-6-deoxy curdlan

1.2.3 Sulfation of chitin and chitosan

Chitin and chitosan sulfates are important derivatives of chitin and chitosan because of their structural similarity to GAGs, leading to potential applications including as blood anticoagulants, antimicrobial agents, metal ion absorbents, and anti-HIV agents. Sulfation of chitin only occurs at the hydroxyl groups (O-3 and O-6) since the acetamide groups are unreactive towards sulfation conditions. Sulfation of chitin has been reported using various sulfating reagents under homogeneous[65] or heterogeneous conditions[66,67]. The regioselectivity studies showed trends similar to those observed with other polysaccharides: sulfation at primary hydroxyl groups (O-6) is highly favored rather than at secondary hydroxyl groups at O-3. For example, selective sulfation at C6 can be achieved by homogeneous sulfation of chitin in DMAc/LiCl at room temperature by using a carefully controlled number of equivalents of SO_3• pyridine.[68] With increased temperature (45 - 75° C) and equivalents of SO_3• pyridine employed, sulfation at C3 is observed as well, with

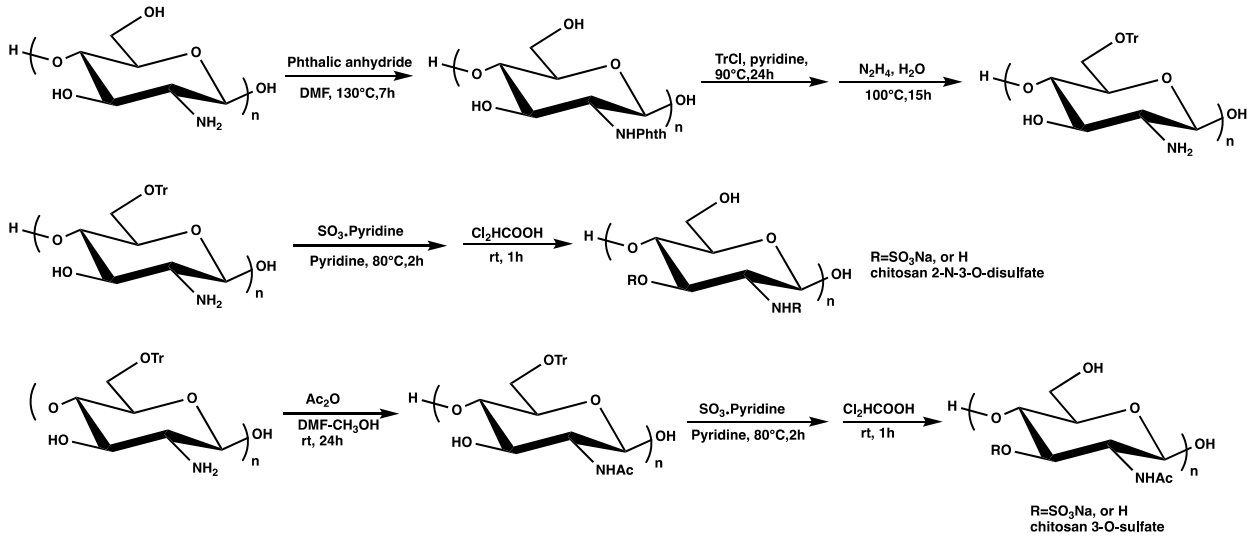
$DS_{sulfate}$ at C3 ranging from 0.48 to 0.91. Sulfation of chitosan occurs not only at the hydroxyl groups (O-3 and O-6) but also at the amine groups because of the high nucleophilicity of the amine nitrogen. Chemo- and regioselective sulfation at hydroxyl and amine groups are accessible by using different solvent systems (Scheme 1.7). Chelation of the amine groups at C2 and the hydroxyl groups at C3 with Cu(II) ions enables regioselective synthesis of chitin 6-*O*-sulfate by subsequent sulfation using SO_3• pyridine in DMF.[69] Sulfation at both O-6 and O-3 is feasible when chitosan is dissolved in dilute acid, since the nucleophilic amine groups get protonated and become no longer nucleophilic. For instance, in the chitosan formic acid-DMF mixture solution, free hydroxyl groups of chitosan react with formic acid to generate formate groups *in situ*, which subsequently react with chlorosulfonic acid to yield chitosan 3,6-*O*-disulfate.[70] The chitosan sulfates obtained showed $DS_{sulfate}$ in the range of 0.65 to 1.0 at O-6 and $DS_{sulfate}$ from 0.29 to 0.61 at O-3, as measured by ^{13}C NMR and elemental analysis. Only the free amine signal was detected in the ^{15}N NMR spectrum, indicating that there was no *N*-sulfation. In 2001, Baumann and Faust reported selective sulfation of amine groups by treatment of chitosan with a rather unreactive sulfating reagent SO_3• trimethylamine complex (its low reactivity is because trimethylamine is a stronger base than pyridine or DMF, thus trimethylamine complex with SO_3 is more stable and less reactive) in alkaline aqueous solution at 65° C.[69] This selective sulfation of amines could be attributed to the higher nucleophilicity of free amine groups than hydroxyl groups in water.



Scheme 1.7 Selective sulfation of chitosan

Protecting group strategies are extremely powerful for regioselective sulfation of chitosan. The free amine groups can be selectively protected by phthalic anhydride to afford unreactive imides, which can be deprotected by hydrazinolysis. Combined with protection of primary hydroxyls as trityl ethers, chitosan sulfates with various sulfate distributions and complete regioselectivity can be prepared. For example, Nishimura et al. reported synthesis of 3-*O*-sulfated chitosan and 2-*N*-3-*O* sulfated chitosan (Scheme 1.8).[71] First, amine groups were protected by reacting chitosan with phthalic anhydride in DMF, followed by reaction with trityl chloride to protect O-6 and deprotection of the imides to liberate amines, affording 6-*O*-trityl chitosan as the key intermediate. The amine groups at C2 and the hydroxyl group at C3 of 6-*O*-trityl chitosan can be sulfated, with subsequent removal of trityl groups in aqueous acid, yielding 2-*N*-3-*O* sulfated chitosan. ^{13}C NMR indicated that sulfation of amines at C2 is nearly quantitative and $DS_{sulfate}$ at O-3 was

measured to be 0.69, while no sulfation at O-6 was detected. 3-*O*-Sulfated chitin was synthesized by a sequence of reactions from 6-*O*-trityl chitosan, including chemoselective acylation of the amine groups, sulfation at O-3, and then removal of trityl groups. ^{13}C NMR confirmed that sulfation occured exclusively at O-3 with $DS_{sulfate}$ 0.44. A similar strategy (phthaloylation of amines, sulfation of hydroxyl groups and deprotection of phthalimide by hydrazinolysis) has also been reported to prepare chitosan 3,6-*O*-disulfate with $DS_{sulfate}$ = 0.97 and 0.79 at C3 and C6 positions, respectively.[69]



Scheme 1.8 Regioselective synthesis of chitosan 3-*O*-sulfate and chitosan 2-*N*-3-*O*-disulfate

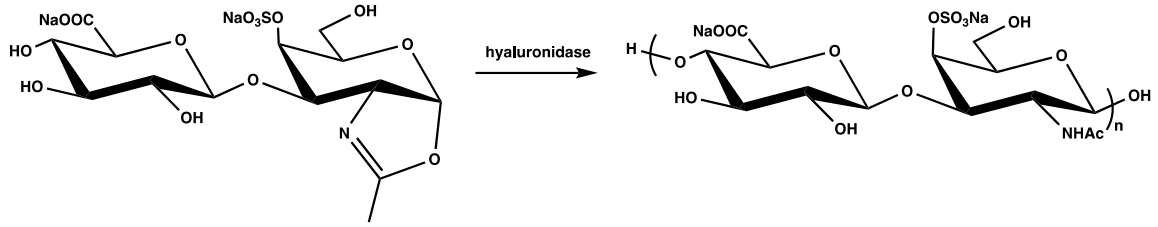
1.3 Chemoenzymatic sulfation of polysaccharides

Many sulfated GAGs are extracted from animal tissues and purified. However, commercial preparation methods usually result in sulfated GAGs with variable molecular weight and sulfation distribution patterns, due to their polydisperse, polycomponent nature.

Furthermore, as a result of the structural similarity between GAGs and their affinity for proteins, contamination also limits the biomedical applications of GAGs. In 2008, oversulfated chondroitin sulfate contaminated heparin and resulted in the death of nearly 100 patients in the US.[72–74] Thus there is a need to produce sulfated GAGs synthetically. Total synthesis has been proved useful in the synthesis of Arixtra, a pentasaccharide mimic for heparin. However, the synthesis of this pentasaccharide requires 50 steps with only 0.1% yield. Chemoenzymatic synthesis of polysaccharide sulfates has merged as a promising alternative to purely chemical synthesis.[75] Furthermore, chemoenzymatic synthesis enables production of GAG libraries which are promising tools for exploring GAG structure activity relationships, and polysaccharide-protein interactions. There are two main synthetic strategies for chemoenzymatic synthesis of polysaccharide sulfates: 1) building polysaccharide chains from disaccharides, and 2) semi-synthesis of polysaccharide sulfates from polysaccharides extracted from organisms.[76]

Employing degradation enzymes and transition state intermediates has been proved to be an effective way for building polysaccharide chains. In 2003, a disaccharide was polymerized with hyaluronidase to prepare a well-defined chondroitin-4-sulfate (Scheme 1.9).[77] The synthesized chondroitin sulfate is perfectly sulfated at C4 position in all GalNAc units, a structure which is not found in nature. The yield of the enzymatic synthesis is 79% with M_n = 12,700. Interestingly, 6-*O*-sulfate GalNAc oxazoline cannot be polymerized to give the corresponding chondroitin sulfate, which is attributed to the low

reactivity of the substrate.



Scheme 1.9 Enzymatic synthesis of chondroitin-4-sulfate

Heparan sulfates with different sulfation patterns can be obtained through chemoenzymatic synthesis.[78–82] Bioengineered synthesis of heparan sulfate usually starts with heparosan, with subsequent enzymatic modifications. Heparosan, which is similar to an unsulfated and unepimerized heparin in structure, comprises a [(→4) β-D-glucuronic acid (GlcA) (1→4) *N*-acetyl-α-D-glucosamine (GlcNAc) (1→)] disaccharide repeating unit.[83,84] Chemoenzymatic synthesis of heparin and its analogues has been reported for several examples. Heparin can be synthesized by chemoenzymatic synthesis starting from *N*-acetylheparosan.[85] *N*-Sulfated heparosan can be prepared by deacylation of *N*-acetylheparosan with alkali, and then *N*-sulfation by SO_3•triethylamine or *N*-sulfotransferase. C5-epimerase coverts GlcA residues to IdoA residues. Subsequent treatment with 2-*O*-sulfotransferase sulfates the O-2 of IdoA. 6-*O*-Sulfotransferase and 3-*O*-sulfotransferase can then introduce sulfate groups to the C6 and C3 position of the GlcNAc or GlcNS units. The sulfate group distribution is carefully controlled by the ratio of substrates to enzymes. By using different combinations of synthetic enzymes, a library of heparan sulfate derivatives can be synthesized for discovering new heparan sulfate with

unique biological functions.[81] For example, heparan sulfates obtained without iduronic acid residues showed the same promising anticoagulation activity as heparin.[86]

1.4. Conclusion

In summary, polysaccharide sulfate derivatives have shown great potential in different applications. Chemical sulfation of polysaccharides is a powerful method to obtain polysaccharide sulfates. Regioselective synthesis can be also accomplished by employing carefully chosen solvent systems and sulfation reagents, or by protecting group strategies. Chemoenzymatic synthesis provides an alternative method to obtain polysaccharide sulfates with very well-defined sulfation distribution, beyond what can be achieved by chemical sulfation of polysaccharides. Despite significant success in sulfation of polysaccharides, convincing analytical methods such as degradation to monomers, and analysis of the monomers have not been applied widely to the sulfated products. Moreover, regioselective synthesis of glycosaminoglycans and their analogues still remains challenging due to their structural complexity. In the future, it will be promising to completely regioselectively synthesize polysaccharide sulfates and develop a comprehensive characterization strategy. Synthesis of GAGs and their analogues with defined sulfate group distributions will also have great potential for biomedical applications.

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Chapter 2: Regioselective chlorination of cellulose esters by methanesulfonyl chloride

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Abstract

Regioselective chlorination of cellulose is challenging due to its low reactivity, the small reactivity differences between cellulosic hydroxyl groups, and the high and diverse reactivity of most common chlorinating agents. Halogenation of cellulose affords useful precursors for subsequent nucleophilic substitution reactions, permitting incorporation of new functionality. Herein we report a simple and efficient pathway for preparation of 6-chloro-6-deoxycellulose esters and their derivatives. Cellulose acetate (degree of substitution (DS) 1.75, CA320S) can be chlorinated by essentially quantitative reaction of the primary alcohol groups with methanesulfonyl chloride (MsCl), yielding 6-chloro-6-deoxy cellulose acetate. Characterization methods including ^{1}H NMR, ^{13}C NMR, FT-IR spectroscopy, and elemental analysis, demonstrated chemo- and regioselective C-6

chlorination. We also demonstrate that chlorinated cellulose acetate is a useful intermediate for displacement reactions with nucleophiles including sodium azide, amines, and thiols to prepare functional cellulose ester derivatives.

2.1 Introduction

Cellulose is an abundant natural polymer, playing a critical structural reinforcement function in all plant cell walls, and is perhaps the most important polysaccharide material in native and modified forms.[1] However, wider application as a renewable biomaterial is hampered by poor cellulose solubility in common solvents and lack of thermoplasticity. Chemical modification is an efficient way to tailor cellulose physicochemical properties (e.g. solubility parameter) and append new functional groups, enabling new applications.[2] Cellulose chemistry still depends heavily on conventional methods like esterification and etherification that use powerful, reactive catalysts, constraining introduction of new functionalities. Increasing demands for renewable biomaterials require more flexible, efficient, and powerful methods to modify cellulose and its derivatives.

Cellulose esters have important applications including in coatings, optical films, drug delivery, composites, and membranes.[3] Regioselective modification of cellulose esters can afford new structures and properties, e.g. through TEMPO oxidation of O-6 primary hydroxyl groups[4], or Staudinger reactions[5,6]. Recently, our group reported synthesis of cationic cellulose esters starting from cellulose or commercial cellulose acetate (CA320S)

by phosphine-catalyzed C-6 bromination and subsequent substitution by aliphatic[7] or aromatic amines[8,9], or phosphines[7]. The ability to convert commercially available, relatively inexpensive cellulose esters into novel, in some cases charged derivatives opens up new vistas in polysaccharide derivative functionality and application.

Replacement of a cellulose hydroxyl group by a nucleophile typically involves two steps: conversion to a good leaving group, and subsequent nucleophilic displacement. Regioselective modifications of cellulose, with few exceptions,[10–12] have been confined to C6, since this primary alcohol has wider approach angles and therefore less steric hindrance to substitution. The *p*-toluenesulfonic acid (tosyl) ester of cellulose is an important precursor to subsequent nucleophilic substitution reactions, due to its availability, hydrolytic stability, and solubility in common organic solvents.[13] Nucleophilic substitution of cellulose tosylate can introduce functional groups including amines,[14–17] heterocycles,[18] and azide.[19–21] Cellulose tosylate can be synthesized by heterogeneous reaction with tosyl chloride in aqueous alkaline solution, or homogeneous reaction in solvents like DMAc/LiCl,[22,23] NaOH-urea aqueous solution,[24] or ionic liquids, e.g. [Amim]Cl[25] or a mixture of BMIMCl and DMI/pyridine.[26] Cellulose reacts with tosyl chloride primarily at the C-6 hydroxyl, displaying nearly complete C-6 regioselectivity up to DS 0.84. At higher DS, tosylation at C2 and C3 is also observed. Competing chlorination can also occur, depending on reaction conditions; organic base and reaction temperature strongly impact the extents of the competing reactions. Chlorination is favored by weak organic bases such

as pyridine or *N,N*-dimethylaniline at high temperature, while tosylation dominates with strong bases, e.g. triethylamine or 4-dimethylaminopyridine, at low temperature.[27,28]

Furuhata et al.[29] reported a direct method for regiospecific, nearly quantitative C-6 bromination of cellulose using triphenylphosphine and N-bromosuccinimide in DMAc/LiBr, affording DS(Br) up to 0.98. This reaction is perhaps the most regioselective in cellulose chemistry.[2] The 6-bromo-6-deoxy entity is a useful intermediate for various substitution reactions, such as reaction with thiols.[30–32] Despite its chemo- and regioselectivity and the high reactivity of bromide towards nucleophilic substitution, bromination has several drawbacks. 6-Bromo-6-deoxycellulose is not completely soluble in common solvents, and the bromination reagents are somewhat costly. Moreover, purification of 6-bromo-6-deoxycellulose is challenging, since Ph_3PO (the by-product of bromination) and residual Ph_3P are difficult to remove completely from the product. For small-molecule brominations,[33] chromatography can be used to separate phosphorus-containing impurities; this is not possible with polysaccharides.

Chlorination could be a less expensive alternative, if regioselective chlorination conditions can be found and if chloride is a sufficiently good leaving group for the desired displacement. Non-specific chlorination of cellulose has been reported with maximum DS 2.8, using classical chlorination reagents such as thionyl chloride[34], phosphoryl chloride[35], and MsCl[36,37] in chloral/DMF; significant loss of DP was also observed. Mild Ph_3P/N-chlorosuccinimide[38,39] successfully chlorinated cellulose, but chlorination was less

regioselective than analogous Furuhata bromination[29], occurring at both C6 and C3 with DS(Cl) up to 1.86. Airoldi et al.[40] reported that treatment with thionyl chloride in DMF afforded completely regioselective cellulose chlorination to 6-chloro-6-deoxycellulose. This built on an earlier report[41] where Vigo and co-workers chlorinated cellulose fibers in heterogeneous fashion with pre-formed chlorodimethylformiminium chloride (from $SOCl_2$/DMF; authors did not analyze regioselectivity). Regioselective chlorination of several other polysaccharides has been achieved. MsCl regioselectively chlorinated amylose[42], pullulan[28], and inulin[43] in DMF with good C-6 regioselectivity (no evidence of chlorination at C2 or C3 observed) and chemoselectivity (mesyl group contents less than 0.05). MsCl can also selectively chlorinate carbohydrate primary hydroxyl groups.[44] In these cases the polysaccharide did not bear other substituents, such as potentially sensitive ester groups. This is significant, since the presence of such substituents conveys solubility (in particular to cellulose), thereby expanding the range of useful solvents. The ability to chlorinate in the presence of ester groups also permits use of inexpensive, high DP cellulose esters as substrates, rather than low DP cellulose sources such as microcrystalline cellulose that are frequently used in cellulose solution modifications (due in part to viscosity limitations). However, a major question is would chloride be an adequate leaving group, in a generally poorly reactive substrate like cellulose, so as to permit displacement by useful nucleophiles like azide, amines, or thiols?

We hypothesize that MsCl can regioselectively chlorinate cellulose esters containing

a substantial DS of unsubstituted 6-OH groups, affording 6-chloro-6-deoxy cellulose esters, without loss of ester groups. We further hypothesize that the chlorinated cellulose esters will undergo subsequent nucleophilic displacement reactions at useful rates and to useful extents, affording cellulose ester derivatives with new functional groups that are otherwise difficult to obtain. If we can realize these hypotheses, we will have developed a more practical, useful route from commercial, high molecular weight, inexpensive cellulose esters to these new cellulose derivative families, including both anionic and cationic polyelectrolytes, avoiding the purification and high expense issues of the exquisitely selective Furuhata bromination route.

Herein, we report attempts to regioselectively chlorinate commercial cellulose acetate with high DS(6-OH). We further attempt to functionalize the resulting chlorinated cellulose acetate by displacement with model nucleophiles as a potential approach towards cellulose-based azides, amines, thiols, and the complete array of amines and amides.[5,42,45–47]

2.2. Experimental

2.2.1. Materials

Cellulose acetate (CA320S, DS(Ac) 1.75, DS(6-OH) 0.49 (calculated from perpropionylated samples by ^{1}H NMR), Mw = 47.6 kDa (measured by SEC), Eastman Chemical Company) was dried under vacuum at 50 °C overnight prior to use (see Tables A2.1 and A2.2, and Figure A2.1 in Supplementary Information for characterization details).

MsCl (99.7+%, Sigma-Aldrich), sodium azide (Fisher), 1-methylimidazole (99+%, Sigma-Aldrich), sodium iodide (Fisher)， 4-dimethylaminopyridine (99%, Fisher), *N, N*-diisopropylethylamine （98+%, Fisher), n-butylamine (99.5%, Sigma-Aldrich), 3-mercaptopropionic acid (99+%, Sigma-Aldrich), 2-mercaptobenzoic acid (97%, Sigma-Aldrich) and propionic anhydride (Sigma-Aldrich) were used as received. Acetone and ethanol were from Fisher Scientific and used as received. *N, N*-Dimethylformamide (DMF, Fisher) and dimethyl sulfoxide (DMSO, Fisher) were kept over 4 Å molecular sieves and stored under dry N_2 until use. Regenerated cellulose dialysis tubing (Fisher, 3500 g/mol MWCO) was used as received.

2.2.2. Measurements

^{1}H and ^{13}C NMR spectra were obtained on a Bruker Avance II 500MHz instrument in DMSO-*d6* or $CDCl_3$ at room temperature, using 64 scans and 15,000 scans, respectively. Chemical shifts are reported relative to the solvent peaks. FT-IR spectra were acquired on a Nicolet 8700 instrument with 128 scans and 4 cm^{-1} resolution. Elemental analysis (EA) was performed by Micro Analysis Inc. using a CE440 analyzer. Carbon, hydrogen and nitrogen contents were determined by flask combustion followed by ion chromatography. Chlorine, iodine and sulfur contents were measured by flask combustion followed by titration. DS values were calculated according to the following equations:

$$DS_{Ac} = \frac{7I_{Ac\ CH_3}}{3I_{cellulose\ backbone}} \quad \text{Eq. 1}$$

$I_{Ac\ CH3}$: integral of acetyl methyl groups

$$DS_{MeIM^+} = \frac{7}{\frac{3I_{cellulose\ backbone+CH_3}}{I_{CH-ring}} - 3} \quad \text{Eq. 2}$$

$I_{cellulose\ backbone+CH3}$: sum of integrals of cellulose backbone hydrogens and 1-methylimidazolium methyl groups

$I_{CH\text{-}ring}$: integral of 1-methylimidazolium aromatic hydrogens

$$DS_{Bu} = \frac{7}{\frac{3I_{cellulose\ backbone+N-CH_2}}{I_{CH_3}} - 2} \quad \text{Eq. 3}$$

$I_{cellulose\ backbone+N\text{-}CH2}$: integral of region including cellulose backbone and methylene groups adjacent to nitrogens of n-butylamino moieties

I_{CH3}: integral of methyl groups of n-butylamino moieties

$$DS_{MBA} = \frac{7I_{CH-ring}}{4I_{cellulose\ backbone}} \quad \text{Eq. 4}$$

$I_{CH\text{-}ring}$: integral of aromatic hydrogens of (2-carboxylphenyl)thioether moieties

$$DS_{Cl} = \frac{\%Cl \times 12.011 \times (6+2 \times DS(Ac))}{\%C \times 35.45} \quad \text{Eq. 5}$$

%Cl and %C are corresponding percentages of chlorine and carbon measured by elemental analysis

DS(Ac) measured by ^{1}H NMR by equation 1

$$DS_S = \frac{\%S \times 12.011 \times (6+2 \times DS(Ac))}{\%C \times 32.06} \quad \text{Eq. 6}$$

%S and %C are corresponding percentages of sulfur and carbon measured by elemental analysis

DS(Ac) measured by ^{1}H NMR by equation 1

2.2.3. Methods

2.2.3.1 Perpropionylation of cellulose acetate (CA320S)

Cellulose acetate (CA320S) was perpropionylated using methods adapted from previous studies.[11,48,49] In a 50 mL round-bottom flask, CA320S was dissolved in pyridine (4 mL) at 80 ˚C under nitrogen. 4-Dimethylaminopyridine (20 mg) and propionic anhydride (4 mL) were slowly added to the solution and stirred at 80 ˚C for 24 h. The reaction mixture was then cooled to room temperature and slowly poured into ethanol (200 mL), followed by filtration to recover the precipitate. The isolated product was then re-dissolved in acetone (10 mL) and re-precipitated in ethanol (100 mL). The sample was collected by filtration and dried under vacuum at 40 °C overnight to yield perpropionylated CA320S. DS (6-OH) in CA320S should be equal to the DS (6-Pr) in perpropionylated CA320S. DS (6-Pr) is calculated according to following equation:

$$DS_{Pr} = \frac{7I_{Pr\ CH_2}}{2I_{cellulose\ backbone}}$$

$I_{Pr\ CH2}$: the signal from methylene groups of propionyl moieties

2.2.3.2 Regioselective chlorination of cellulose acetate

In a 100 mL round-bottom (RB) flask CA320S (0.5 g, 2.1 mmol) was dissolved in 20 mL of anhydrous DMF at 75 °C. MsCl (1.6 mL, 10 equiv per AGU) was added dropwise to the solution. The reaction mixture was kept at 75 °C for 3 h under N_2 as the solution turned from colorless to yellow. It was slowly poured into 300 mL of deionized water, followed by filtration. The crude product was re-dissolved in acetone (5 mL) and re-precipitated in water (100 mL). The precipitate was recovered by filtration, washed extensively with water and ethanol, and vacuum dried overnight at 40 °C to yield (6-chloro-6-deoxy)-co-(6-O-acetyl)-CA320S (6-ClCA320S). ^{13}C NMR (500 MHz, DMSO-*d6*): 21.05 (O-(C=O)-$\underline{C}H_3$), 44.02 (<u>C-6</u>-Cl), 62.64 (<u>C-6'</u>-O-Ac), 71.74-79.91 (C2, C3, C4 and C5), 99.65 (C1), 103.43 (C1'), 161.30 (O-(<u>C</u>=O)-H), 169.47-170.81 (O-(<u>C</u>=O)-CH_3). EA: %C 46.16, %H 5.40, %Cl 7.28, %N 0.00, %S 0.58. DS calculated by EA: DS(Cl) = 0.51. Maximum possible DS(Mesyl) calculated by EA (assuming all S comes from mesyl groups): DS(Ms) = 0.04. Yield: 421 mg (81.0%).

2.2.3.3 Synthesis of (6-azido-6-deoxy)-co-(6-O-acetyl)-CA320S (6-N3CA320S)

6-ClCA320S (200 mg, 0.82 mmol) was dissolved in 10 mL of anhydrous DMSO in a 100 mL RB flask, then NaN_3 (159 mg, 3 equiv per AGU, 5.88 equiv per Cl) was added to the flask. The reaction mixture was heated to 80 °C and stirred for 24 h under N_2. The solution was poured into 200 mL deionized water and the precipitate was collected by filtration. The product was washed with deionized water and ethanol before vacuum drying at 40°C, affording (6-azido-6-deoxy)-co-(6-O-acetyl)-CA320S (6-N_3CA320S). ^{13}C NMR

(500 MHz, DMSO-*d6*): 21.02 (O-(C=O)-CH$_3$), 50.25 (C-6-N$_3$), 62.59 (C-6'-O-Ac), 71.82-79.36 (C2, C3, C4 and C5), 99.61 (C1), 103.15 (C1'), 161.58 (O-(C=O)-H), 169.43-170.77 (O-(C=O)-CH$_3$). EA: %C 44.84, %H 5.09, %N 7.28, %Cl 0, %S 0.36. DS calculated by EA: DS(N$_3$) 0.44. Yield: 182 mg (90 %).

2.2.3.4 Synthesis of (6-(1-methyl-3-imidazolio)-6-deoxy)-co-(6-O-acetyl)-CA320S (6-MeIMCA320S)

In a 100 mL RB flask, 200 mg of 6-ClCA320S (0.82 mmol) was dissolved in 10 mL of anhydrous DMSO. NaI (245 mg, 2 equiv per AGU, 3.92 equiv per Cl) and 1-methylimidazole (1.3 mL, 20 equiv per AGU, 39.2 equiv per Cl) were added to the flask. The solution was stirred at 80 °C for 48 h under N_2. The product was collected by dialysis against ethanol for 3 days, and then against 0.9 % sodium chloride solution for 3 days, followed by freeze-drying. ^{1}H NMR (500 MHz, DMSO-*d6*): 1.85-2.15 (O-(C=O)-CH$_3$), 2.75-5.60 (cellulose backbone and N-CH$_3$), 7.32-7.79 (N-CH=CH-N-CH$_3$ and N-CH=CH-N-CH$_3$), 9.12 (N=CH-N-CH$_3$); ^{13}C NMR (500 MHz, DMSO-*d6*): 20.62, 24.78 (O-(C=O)-CH$_3$), 35.75 (N-CH$_3$), 49.00 (C-6-N), 62.98 (C-6'-O-Ac), 71.75-80.40 (C2, C3, C4 and C5), 98.95 (C1), 102.60 (C1'), 123.03 (N-CH=CH-N-CH$_3$ and N-CH=CH-N-CH$_3$), 138.03 (N=CH-N-CH$_3$), 169.39, 170.28, 175.05 (O-(C=O)-CH$_3$). DS by ^{1}H NMR: DS(MeIM$^+$) 0.41, DS(Ac): 1.24. Yield: 195 mg (72 %).

2.2.3.5 Synthesis of (6-butylamino-6-deoxy)-co-(6-OH) cellulose (6-BuNCellulose)

6-ClCA320S (200 mg, 0.82 mmol) was dissolved in 10 mL of anhydrous DMSO in a

100 mL RB flask. NaI (245 mg, 2 equiv per AGU, 3.92 equiv per Cl) and n-butylamine (1.21 mL, 15 eq per AGU, 29.41 equiv per Cl) were slowly added to the flask. The reaction mixture was heated to 70°C and stirred at that temperature for 24 h. The solution was slowly poured into 150 mL of 50:50 (v/v) ethanol/deionized water to precipitate the product, followed by filtration. The product was extensively washed with deionized water and ethanol before vacuum drying at 40°C, affording 6-BuNCellulose. ^{1}H NMR (500 MHz, DMSO-*d6*): 0.83-0.94 (N-$CH_2CH_2CH_2\underline{CH_3}$), 1.25-1.42 (N-$CH_2CH_2\underline{CH_2}CH_3$), 1.50-1.64 (N-$CH_2\underline{CH_2}CH_2CH_3$) and 2.82-5.00 (cellulose backbone and N-$\underline{CH_2}CH_2CH_2CH_3$); ^{13}C NMR (500 MHz, DMSO-*d6*): 13.46 (N-$CH_2CH_2CH_2\underline{C}H_3$), 19.26 (N-$CH_2CH_2\underline{C}H_2CH_3$), 27.38 (N-$CH_2\underline{C}H_2CH_2CH_3$), 47.10-47.40 (<u>C-6-N</u> and N-$\underline{C}H_2CH_2CH_2CH_3$), 60.31 (<u>C-6-OH</u>), 69.29-81.77 (C2, C3, C4 and C5) and 102.83 (C1). DS calculated by ^{1}H NMR: DS (Bu) 0.39. EA: %C 44.75, %H 7.30, %N 2.32, %Cl 2.59 and %I 0.00. DS obtained by EA: DS (Bu) 0.37. Yield: 112 mg (63.6%).

2.2.3.6 Synthesis of (6-(2-carboxyphenyl)thio-6-deoxy)-co-(6-O-acetyl)-CA320S (6-MBACA320S)

In a 100 mL RB flask, 6-ClCA320S (200 mg, 0.82 mmol) was dissolved in 10 mL of anhydrous DMSO. NaI (245 mg, 2 equiv per AGU, 3.92 equiv per Cl), *N, N*-diisopropylethylamine (4.3 mL, 30 equiv per AGU), and 2-mercaptobenzoic acid (2.52 g, 20 equiv per AGU, 39.2 equiv per Cl) were added to the flask. The solution was stirred at 80°C for 24 h under N_2. The product was collected by dialysis against ethanol for 3 days,

and then against deionized water for 2 days, followed by filtration. The product was washed extensively with ethanol before drying under vacuum at 40°C to yield 6-MBACA320S. ^{1}H NMR (500 MHz, DMSO-*d6*): 1.60-2.20 (O-(C=O)-$\underline{CH_3}$), 2.77-5.66 (cellulose backbone) and 6.95-8.15 (phenyl ring); ^{13}C NMR (500 MHz, DMSO-*d6*): 20.57 (O-(C=O)-$\underline{C}H_3$), 32.66 ($\underline{C\text{-}6}$-S), 62.52 ($\underline{C\text{-}6'}$-O-Ac), 71.27-78.57 (C2, C3, C4 and C5), 98.99 (C1'), 102.29 (C1), 123.76, 125.66, 130.79, 132.35, 140.91 (phenyl ring) and 167.52-170.30 (O-($\underline{C}$=O)-CH_3 and ($\underline{C}$=O)-OH). DS by ^{1}H NMR: DS(2-MBA) = 0.49, DS(Ac) = 1.56. Yield: 168 mg (67.7%).

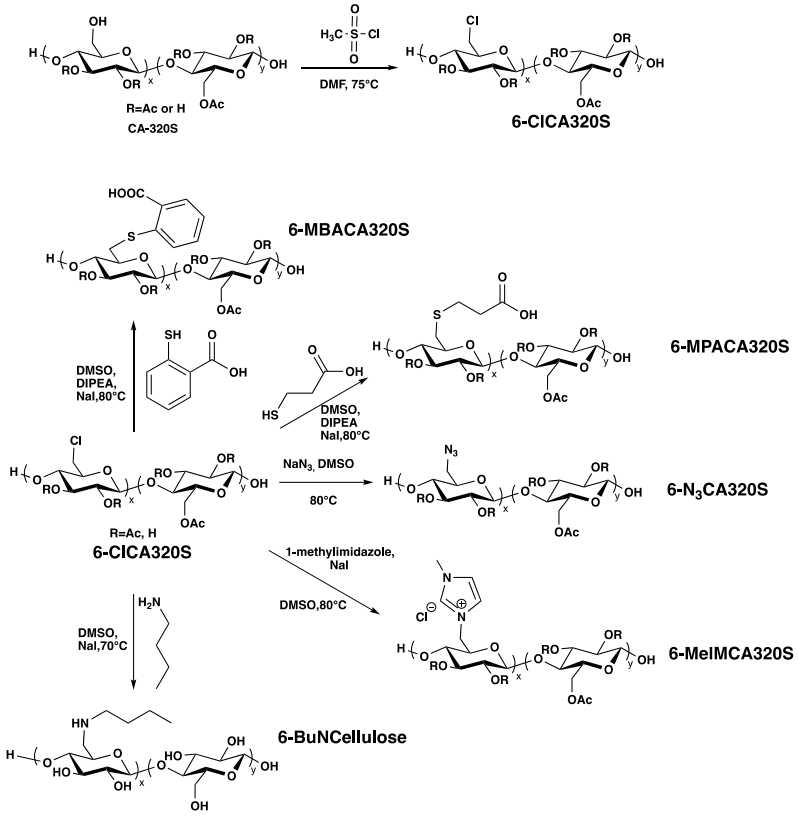
2.2.3.7 Synthesis of (6-(2-carboxyethyl)thio-6-deoxy)-co-(6-O-acetyl)-CA320S (6-MPACA320S)

6-ClCAS (200 mg, 0.82 mmol) was dissolved in 10 mL anhydrous DMSO in a 100 mL RB flask. NaI (245 mg, 2 equiv per AGU, 3.92 equiv per Cl), *N, N*-diisopropylethylamine (4.3 mL, 30 equiv per AGU) and 3-mercaptopropionic acid (1.42 mL, 20 equiv per AGU, 39.2 equiv per Cl) were added to the flask. The solution was heated to 80°C and kept at that temperature for 24 h under N_2. The product was isolated by dialysis against deionized water for 3 days, followed by filtration, before vacuum drying at 40°C to afford 6-MPACA320S. ^{1}H NMR (500 MHz, DMSO-*d6*): 1.61-2.15 (O-(C=O)-$\underline{CH_3}$), 2.52-2.61 (-S-CH_2-$\underline{CH_2}$-COOH), 2.76-2.95 (-S-$\underline{CH_2}$-CH_2-COOH) and 2.96-5.27 (cellulose backbone); ^{13}C NMR (500 MHz, DMSO-*d6*): 20.62 (O-(C=O)-$\underline{C}H_3$), 32.50-33.57 ($\underline{C\text{-}6}$-S, -S-$\underline{C}H_2$-CH_2-COOH and -S-CH_2-$\underline{C}H_2$-COOH), 62.28 ($\underline{C\text{-}6'}$-O-Ac), 71.29-79.77 (C2, C3,

C4 and C5), 99.37 (C1'), 102.91 (C1), 169.04-171.00 (O-(C=O)-CH_3) and 172.72 ((C=O)-OH). EA: %C 45.77, %H 5.22, %N 0.00, %S 4.35, %Cl 3.31 and %I 0.00. DS obtained by combination of EA and ^{1}H NMR: DS(MPA) 0.36 DS(Ac) 1.55. Yield: 147 mg (64%).

2.3 Results and Discussion

Initially we explored chlorination of a commercially available random copolymer, cellulose acetate (CA320S, DS(Ac) 1.75), with its high content of primary hydroxyl groups. By ^{1}H NMR analysis of a perpropionylated sample (Figure A2.1), DS(6-Pr) was 0.49, so (Table A2.1) DS(6-Ac) in the original CA320S was 0.51 and DS(6-OH) 0.49.[8,11,48]



Scheme 2.1 Regioselective chlorination of CA320S and subsequent displacement by azide, amines and thiols.

2.3.1 Chlorination of CA320S by methanesulfonyl chloride

MsCl is useful for regioselective chlorination of carbohydrates and polysaccharides. However, there were many potential impediments to MsCl chlorination of CA; chemoselectivity (whether high DS(mesyl) would be introduced, possible reactivity of acyl groups), regioselectivity, side reactions (e.g. deacylation, cross-linking), and whether chain degradation would be significant in the presence of the co-product acids (e.g. HCl, CH_3SO_3H). We reacted CA320S with MsCl in DMF (75 °C, 3 h, Scheme 2.1). In the ^{13}C NMR spectrum of the product (Figure 2.1), a new peak at 44 ppm consistent with Cl-bearing C-6[42] was clear evidence of the hypothesized regioselective chlorination. The product FT-IR spectrum (Figure A2.3) also shows C-Cl stretch at 720 and 753 cm^{-1}, confirming successful chlorination. DS (Cl) measured by EA was 0.51, vs. the starting CA-320S DS (6-OH) of 0.49 as measured by ^1H NMR, consistent with quantitative C-6 chlorination, with off-target chlorination minimal at most. Sulfur and nitrogen content were found to be low (0.58 %) and nonexistent (0.00 %), respectively, indicating that DS (mesylate) did not exceed 0.04, and that no DMF by-products were present. No mesylate groups were detected by ^1H NMR (sharp peak at 3-4 ppm for $–OSO_2-C\underline{H}_3$), ^{13}C NMR (38 ppm for $–OSO_2-\underline{C}H_3$), or FT-IR spectra (characteristic -O-SO_2-R bands ca. 1350 and 1175 cm^{-1}),[28] confirming chemoselective chlorination and no more than minimal formation of mesylate groups (we cannot rule out the presence of minor sulfur-containing impurities in

the product as the possible source of S). The mechanism of alcohol chlorination by MsCl in DMF has been previously elucidated.[27,37] First, MsCl reacts with DMF to form the *O*-(methanesulfonyl)-*N, N*-dimethylformimidium chloride salt, $CH_3SO_3CH{=}NMe_2^+Cl^-$ (Scheme 2.2). This intermediate reacts with the alcohol, affording, in the case of cellulose, an *N*, *N*-dimethylformiminium chloride, Cell-O-$CH{=}NMe_2^+Cl^-$, followed by displacement of DMF by chloride ion, to yield chlorinated cellulose acetate.This last displacement involves an S_N2 reaction, thereby ensuring the observed C-6 selectivity (displacement at

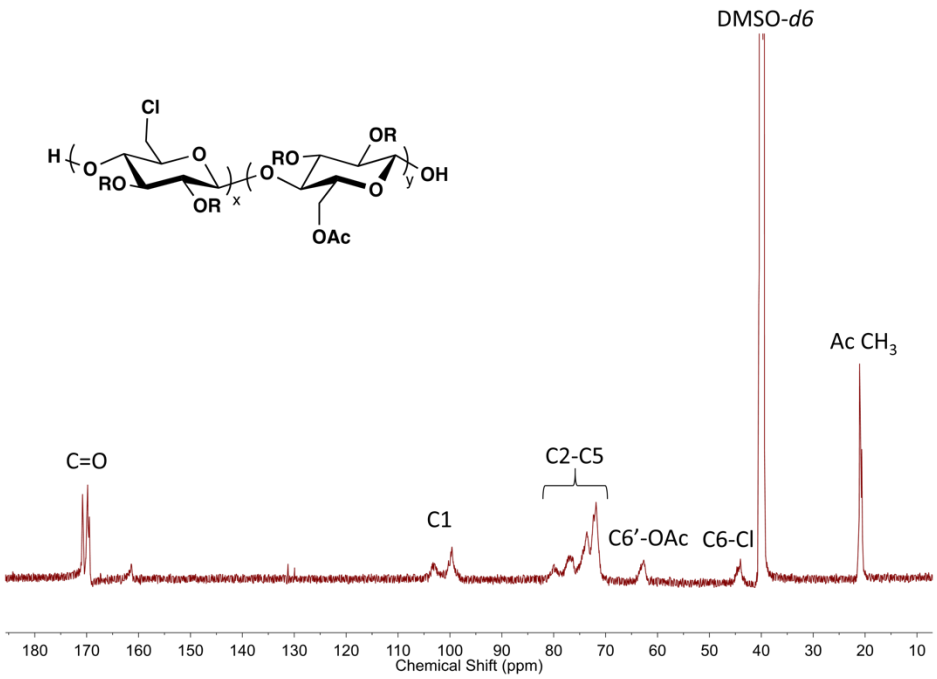


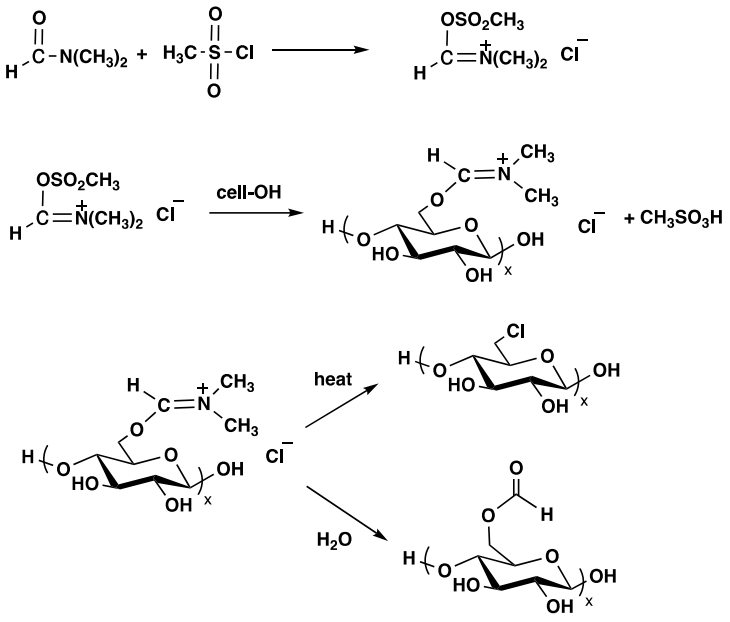
Figure 2.1 ^{13}C NMR spectrum of 6-ClCA320S.

C-2 or C-3 being disfavored both by restricted approach angles and the required glucose ring inversion). The observation of a small amount of appended formate groups, (160 ppm by ^{13}C NMR (formate C=O), 7.0-7.5 ppm by ^{1}H NMR (-CH=O), Figure A2.2), further

supports the reaction mechanism, since formate groups result from hydrolysis of unreacted *N*, *N*-formiminium chloride intermediate, presumably during aqueous work-up.[41] Product DS(Ac) was 1.75, identical with that of starting CA and confirming that little or no deacylation had occurred. Others observed significant DP loss when using MsCl to chlorinate cellulose in chloral/DMF, so it is gratifying that our chlorinated product (SEC, Table A2.2) showed no or minimal depolymerization, supporting the selective and mild nature of this method. Product 6-ClCA320S showed good solubility in polar aprotic solvents (DMF, DMSO, DMAc; in fact similar solubility to starting CA320S), indicating that no or little cross-linking had occurred. Compared with the exquisitely selective Furuhata bromination using PPh_3/NBS, this chlorination method has several advantages: MsCl is a relatively inexpensive reagent; no phosphines are required; and product separation and purification are facile, with little side product formation (the few formates are readily removed by hydrolysis[50], and/or in the subsequent displacement reaction).

2.3.2 Reaction with azide (synthesis of 6-N_3CA320S)

The key remaining question was whether the chlorinated cellulose esters were sufficiently reactive towards subsequent nucleophilic displacements, given the lower reactivity of alkyl chlorides than alkyl bromides. We first tested reactivity towards the highly nucleophilic azide. It is well established that polysaccharides containing leaving groups including halides and tosylate can undergo azide displacement quantitatively at C-



Scheme 2.2 Mechanism of cellulose ester chlorination

6.[5,42] Moreover, azide is a promising functional group for further reduction to amine or amide,[5,47,51] or as a partner in Huisgen click reactions[52,53] to append new functionalities[19,54]. Reaction of 6-ClCA320S with NaN_3 (DMSO, 24 h, similar or identical reaction conditions and equivalents of sodium azide compared with starting from brominated or tosylated cellulose derivatives[5,8,21]) afforded 6-N_3CA320S, as confirmed by FT-IR, ^{13}C NMR, and ^{1}H NMR spectra. The FT-IR spectrum (Figure 2.2) displayed a strong azide absorption at 2111 cm^{-1} and an ester carbonyl absorption at 1752 cm^{-1}, confirming successful introduction of azide groups. By ^{13}C NMR (Figure 2.3), the C6-Cl peak at 44 ppm was absent while a new resonance at 50 ppm was assigned as the azide-bearing C6[5]. EA is consistent with DS (N_3) 0.44, with no Cl found, confirming complete conversion of C6-Cl, mostly to C6-N_3. Chlorinated cellulose esters were found to be as reactive towards the

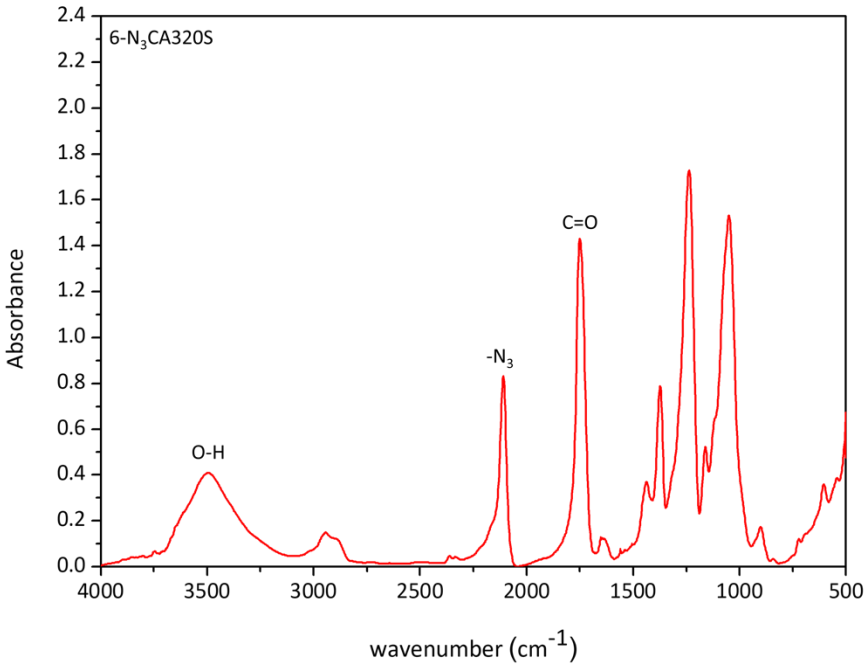


Figure 2.2 FT-IR spectrum of 6-N_3CA320S.

strong nucleophile azide as brominated or tosylated cellulose derivatives.[5,21] Failure to detect any Cl further supports the hypothesis that chlorination by MsCl is regioselective at C6, since azide displacement of C2 or C3 chlorides is unlikely due to issues interfering with S_N2 chemistry at those sites as previously discussed, so any C-2 or C-3 chlorides that were present in the chlorination product should remain in the azide and be detected by EA. A small amount of sulfur was detected in the product by EA (0.36%, corresponding to maximum possible DS(mesyl) of 0.03). This may suggest either a small amount of mesylation at C2 or C3 OH, or a small amount of sulfur-containing impurities that are difficult to remove. DS(Ac) of the product was 1.75 by ^{1}H NMR (Figure A2.4), indicating little or no deacylation during azide displacement. No DP loss was evident after azide

displacement (SEC, Table A2.2). Successful synthesis of 6-azido-6-deoxycellulose derivatives creates the potential to synthesize cellulose derivatives bearing 6-amine or 6-amide groups by Staudinger or other reduction methods; we have demonstrated successful Staudinger reductions to amines and amides that also bear acetate esters on the secondary OH groups (substrates prepared through a route starting with Furuhata bromination) on 6-OH-containing polysaccharides including cellulose[5], curdlan[46,47,51], and pullulan[45].

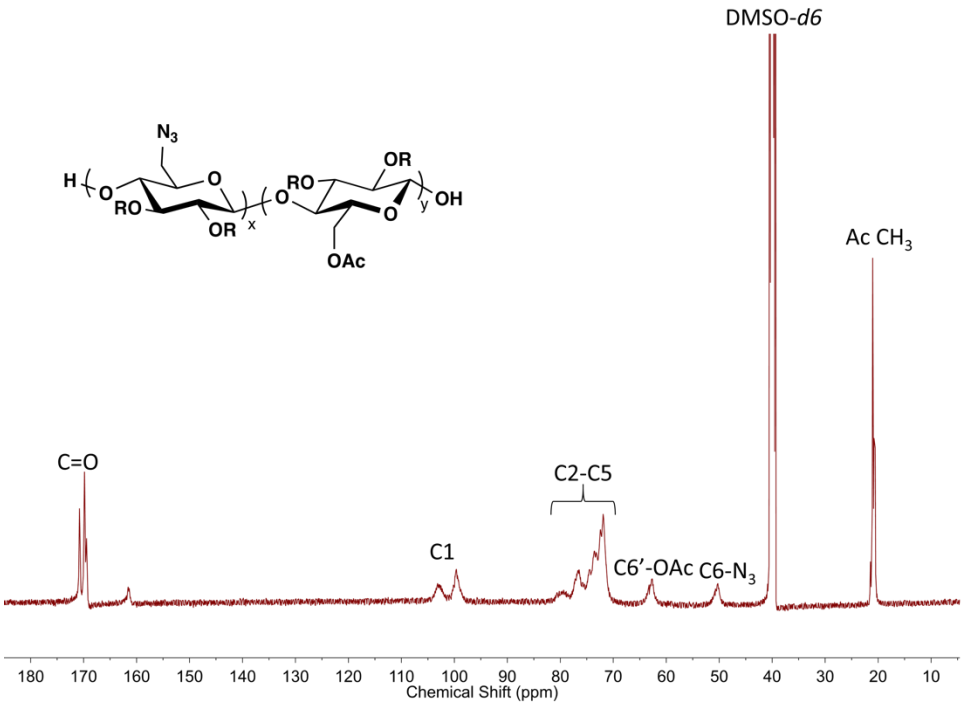


Figure 2.3 ^{13}C NMR spectrum of 6-N_3CA320S.

2.3.3 Reaction with amines

After complete conversion to 6-N_3CA320S by the highly nucleophilic azide, we wished to explore the reactivity of the C6-Cl towards weaker amine nucleophiles. The aromatic amine, 1-methylimidazole, was used as a model for the potential of 6-chloro-6-

deoxycellulose esters as gateways to synthesis of cationic cellulose ester derivatives, which have intriguing potential in biomedical applications.[7–9,18,55] A simpler synthesis strategy for pure, regioselectively substituted, cationic cellulose esters can facilitate study of the structure-property relationships for important applications like tight-junction opening (oral protein delivery)[56,57] or DNA/RNA polyplex formation[58,59]. 6-ClCA320S was initially treated with 1-methylimidazole (20 eq per AGU) in DMSO (80 °C, 48 h). The water-insoluble product DS ($MeIM^+$) was as low as 0.03, vs. the water-soluble product with DS ($MeIM^+$) 0.46 obtained from 6-BrCA320S[8]. Longer reaction time (72 h) or increased molar equivalents of 1-methylimidazole (to 40-50/AGU) did not increase DS ($MeIM^+$). To improve conversion, NaI was added to generate concentrations of the 6-deoxy-6-iodocellulose acetate intermediate *in situ*, to take advantage of better leaving group potential of iodide.[60] Upon addition of 2 equiv NaI/AGU, DS($MeIM^+$) increased significantly from 0.03 to 0.41, affording a water-soluble polymer, and conversion reached a more satisfying 84 %. The product (Figure 2.4) shows new resonances at 7.5-9.5 ppm (^{1}H NMR) from aromatic protons of the 1-methylimidazolium substituent, and a sharp peak at 4 ppm from the N-methyl group, indicating successful 1-methylimidazolium incorporation. New ^{13}C NMR (Figure A2.5) resonances appeared at 50.25 ppm, assigned to the imidazolium-substituted C-6, and at 120.03 and 138.03 ppm, assigned to the imidazolium aromatic carbons. FT-IR (Figure A2.6) shows the C-N stretch at 1567 cm^{-1} and the ester C=O stretch at 1740 cm^{-1}, further confirming the postulated imidazolium

displacement. 6-MeIMCA exhibited excellent organic solubility, e.g. in DMF, DMSO and DMAc, and water solubility (> 40 mg/mL), also advantageous for biomedical applications.

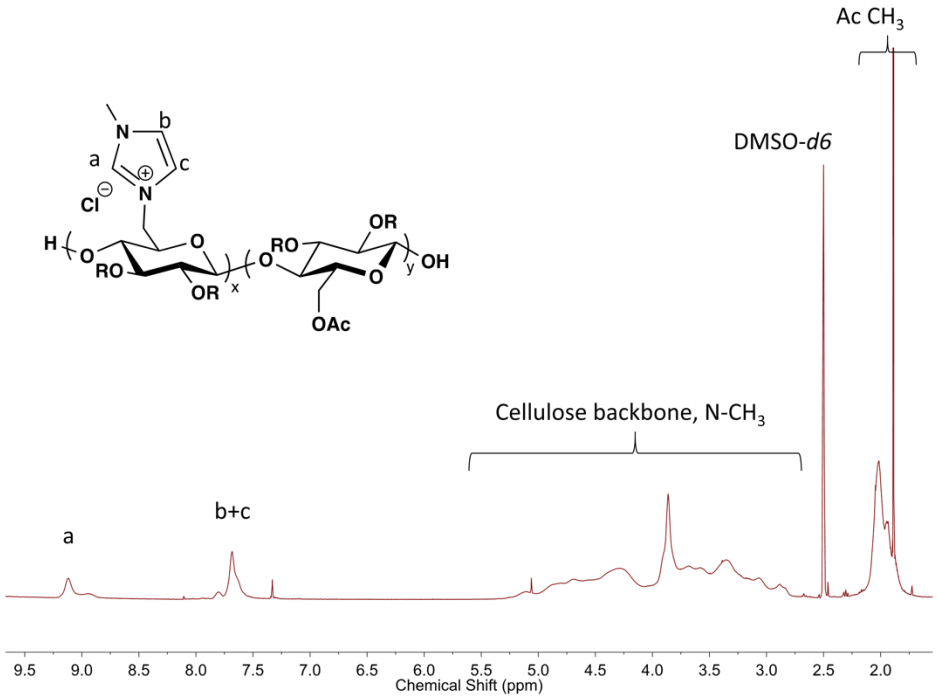


Figure 2.4 ^{1}H NMR spectrum of 6-MeIMCA320S.

We next examined 6-Cl reactivity with a model primary aliphatic amine, n-butylamine, and sodium iodide (DMSO, 80°C, 24h) affording 6-BuNCellulose. Three new aliphatic proton resonances (^{1}H NMR, Figure 2.5) appear between 0.8 and 1.7 ppm from the n-butylamino substituent, indicating successful amine displacement. A new ^{13}C resonance (Figure 2.6) at 47 ppm is attributed to N-substituted C6 and the BuN methylene adjacent to nitrogen. Three new ^{13}C resonances between 13 - 28 ppm are assigned to two methylenes and one methyl group of the n-butylamino moiety. DS(Bu) was 0.39 by ^{1}H NMR, and 0.37 by EA, showing reasonable agreement, and good conversion (76-80%) in this primary

amine substitution. EA showed that the product contained DS(Cl) of 0.14, with no iodine content. This suggests that the *in situ* generated 6-deoxy-6-iodo intermediate is substituted quantitatively by the primary aliphatic amine. Interestingly, complete deacylation was also observed, confirmed by disappearance of acetyl proton resonances. Deacylation of cellulose esters by primary amines has been previously observed by our group[5,61] and others[62].

We attempted reactions of 6-ClCA320S with a model secondary aliphatic amine, diethylamine. However, the products had low DS(NEt_2) ($\leq$ 0.05), even with excess NaI or Et_2NH, or longer reaction times. Reaction of the less reactive alkyl chloride, even in the presence of iodide, with secondary amines seems more difficult.

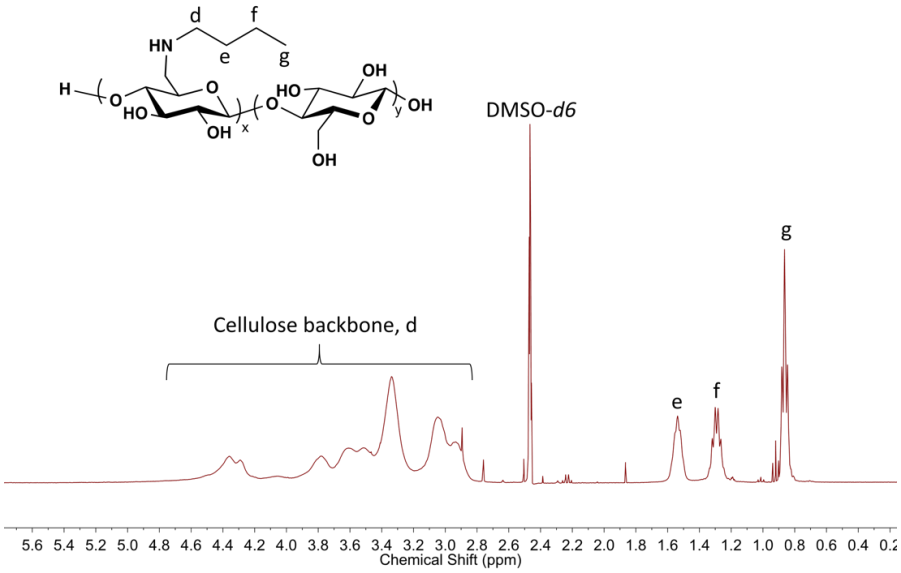


Figure 2.5 ^{1}H NMR spectrum of 6-BuNCellulose

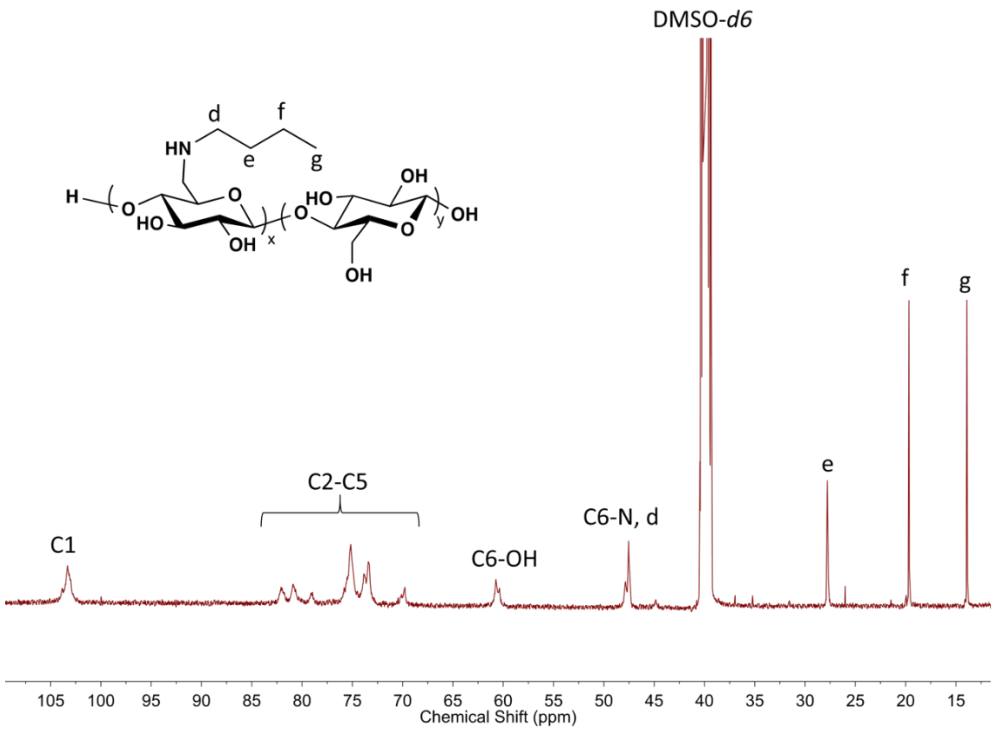


Figure 2.6 ^{13}C NMR spectrum of 6-BuNCellulose

2.3.4 Reaction with thiols

Reaction of 6-ClCA320S with the model aromatic thiol, 2-mercaptobenzoic acid[31] in the presence of *N,N*-diisopropylethylamine (DIPEA, to deprotonate the thiol; NaI, DMSO, 80°C, 24 h) afforded the desired 6-MBACA320S. By ^{1}H NMR (Figure 2.7) the product shows new resonances between 6.9 - 8.1 ppm, assigned to the new thioether aromatic protons. The ^{13}C NMR C6-Cl resonance (Figure A2.7) at 44 ppm was absent, while a new resonance at 32 ppm was assigned to C-6 bound to sulfur. Aromatic carbon (124 and 141 ppm), ester carbonyl, and carboxyl carbonyls (167 - 170 ppm) further confirmed successful aromatic thioether introduction. DS(MBA) was 0.49 (^{1}H NMR), indicating quantitative conversion to the thioether. DS(Ac) was 1.56 by ^{1}H NMR (vs. starting 6-ClCA320S DS

(Ac) 1.75), indicating a small amount of deacylation by the thiolate anion.

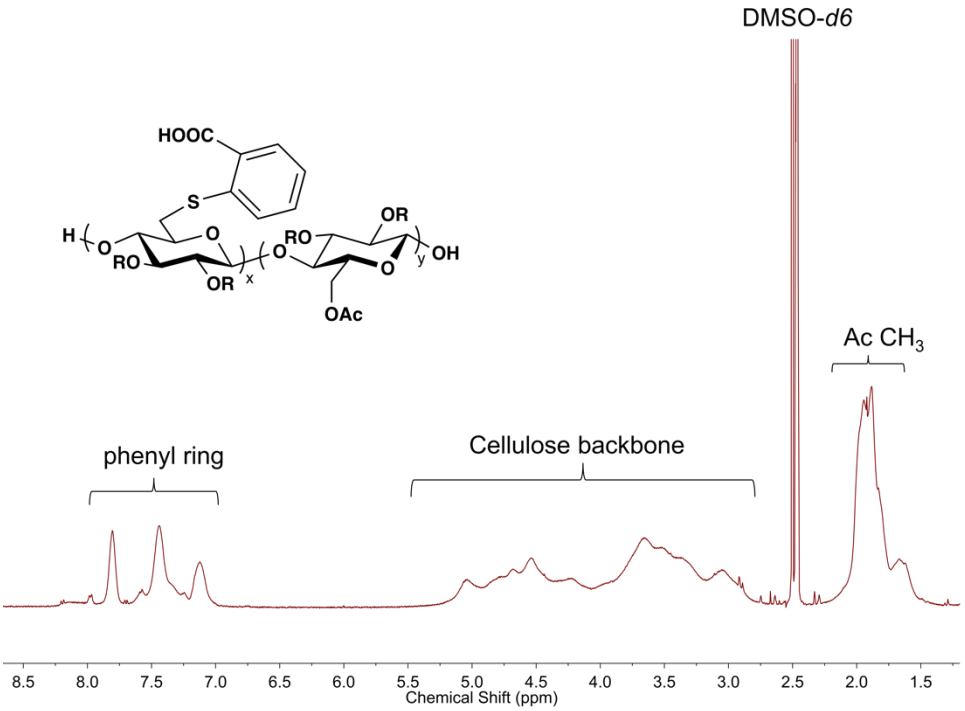


Figure 2.7 ^{1}H NMR spectrum of 6-MBACA320S.

C6-Cl reactivity with the model aliphatic thiol 3-mercaptopropionic acid was of particular interest since successful displacement would append a valuable carboxyl group.[61,63] 6-ClCA320S was treated with 3-mercaptopropionic acid, NaI, and DIPEA in DMSO at 80°C for 24 h. Two new product ^{1}H NMR resonances (Figure A2.8) between 2.6 - 2.8 ppm were assigned as the thioether methylene groups. New ^{13}C NMR resonances (Figure 2.8) (31 - 33 ppm) were assigned as the overlap of S-bound C-6 and the thioether methylenes. The carboxyl carbonyl resonated at 172 ppm. Peak assignments were confirmed by a 2D HSQC experiment (Figure A2.9). DS(MPA) was 0.36 by a combination of EA and ^{1}H NMR integration, indicating approximately 70% conversion of 6-Cl to

thioether. Lower conversion using 3-mercaptopropionic acid versus 2-mercaptobenzoic acid is likely explained by mercaptan acidities.[31] 2-Mercaptobenzoic acid has mercaptan pKa = 8.88, while the mercaptan pKa of 3-mercaptopropionic acid is 10.20.[64] The resulting higher extent of dissociation of 2-mercaptobenzoic acid than 3-mercaptopropionic acid in the presence of the DIPEA base (pKa 10.75) leads to higher displacement conversion, since deprotonated thiols are far more reactive nucleophiles than their conjugate acids.

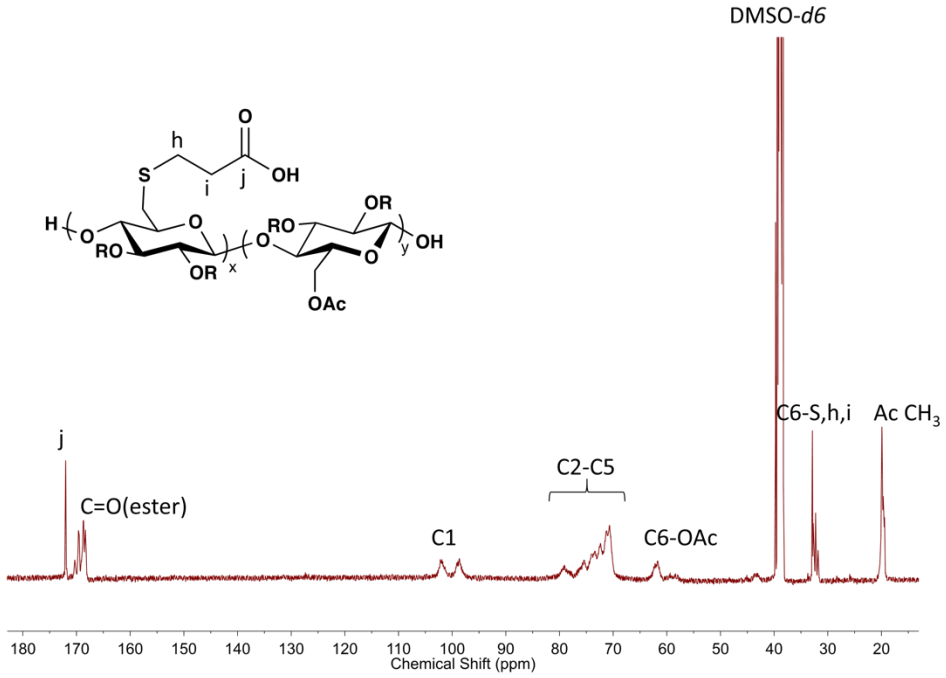


Figure 2.8 ^{13}C NMR spectrum of 6-MPACA320S

2.4. Conclusions

We have developed a facile, efficient method for chlorination of cellulose esters using MsCl. This chlorination reaction is highly regioselective for C6, and highly chemoselective despite the presence of ester groups, with negligible side reactions (mesylation, formation

of formate groups), while preserving DP. We illustrate the utility of the resulting 6-chloro-6-deoxy copolymers as intermediates for appending new functional groups, affording cellulose ester derivatives with well-defined structures. The chlorinated cellulose esters undergo displacement with strong nucleophiles (NaN_3) quantitatively at C6, affording useful precursors for cellulose-based 6-amino-6-deoxy or 6-amido-6-deoxy derivatives. The C-6 chloride is significantly less reactive than 6-bromo substituents towards weaker nucleophiles (e.g. aromatic amines like 1-methylimidazole), resulting in low displacement conversion. However, formation of 6-iodo-6-deoxy cellulose esters *in situ* by adding NaI improves conversion significantly, following the Curtin-Hammett principle[60], e.g. affording higher DS (imidazolium), thereby providing a cationic, water-soluble polyelectrolyte. This strategy afforded high conversions (>75%) in reactions of these regioselectively chlorinated cellulose esters with various nucleophiles including aliphatic amines, aliphatic thiols, and aromatic thiols. Secondary amines were less useful nucleophiles. This method has strong potential for practical preparation of chlorinated cellulose ester derivatives due to its ease of separation, low cost reagents, and sufficient reactivity of chlorinated cellulose esters with nucleophiles. With subsequent chloride displacement, this provides simple, efficient, selective, and practical access to a wide variety of cellulose ester derivatives, including cationic and anionic derivatives, permitting investigators to bypass the expense and purification issues inherent in the otherwise excellent Furuhata bromination. It is likely to be suitable for chlorination of other

polysaccharide esters that contain an adequate DS of free 6-OH groups, and it is also likely that other, valuable nucleophiles can be thus appended regioselectively to the polysaccharide chain. The ready availability of cellulose-based polyelectrolytes made possible by this chemistry should benefit a number of applications, enabling detailed structure-property studies, including examples like amorphous solid dispersion[63,65] and tight junction opening[56,57].

Acknowledgements

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Chapter 3: Summary and Future Work

3.1 Summary

This thesis describes an efficient and practical method for chlorination of cellulose esters by using MsCl. This chlorination is regioselective for C6, and highly chemoselective, as confirmed by characterization methods including ^{1}H NMR, ^{13}C NMR, FT-IR spectroscopy, and elemental analysis. Even in the presence of labile ester groups, only negligible amounts of side reactions (mesylation, formation of formate groups) occur. The reactions are mild, incurring virtually no loss of DP.

The reactivity of the C-6 chloride is further examined by employing a wide variety of nucleophiles, including azides, aromatic amines, aliphatic amines, aliphatic thiols, and aromatic thiols. Our results indicate that chlorinated cellulose esters are useful intermediates for appending new functional groups by nucleophilic displacement reactions. The chlorides of 6-chloro-6-deoxy groups on polysaccharide copolymers can be substituted by strong nucleophiles (e.g. azides) quantitatively at C6, yielding useful precursors for cellulose-based amines or amides. The C-6 chloride is not as reactive as a C-6 bromide towards weaker nucleophiles, such as 1-methylimidazole, resulting in low displacement conversion. Adding NaI to generate 6-iodo-6-deoxy cellulose esters *in situ* significantly improves reaction conversion with weak nucleophiles, affording a cationic, water-soluble

polyelectrolyte in the case of 1-methylimidazole displacement. This strategy is also employed in reactions of chlorinated cellulose esters with other nucleophiles, including aliphatic amines, aliphatic thiols, and aromatic thiols, with high displacement conversions observed.

This regioselective chlorination method has great potential for practical preparation of novel cellulose ester derivatives, owing to the ease of separation, low-cost reagents, and sufficient reactivity of chlorinated cellulose esters with nucleophiles. It is likely that this chlorination method is suitable for chlorination of other polysaccharides and their derivatives that contain a substantial DS of free 6-OH groups. Anionic and cationic cellulose ester derivatives prepared by this method facilitate detailed structure activity relationship studies for numerous applications, including amorphous solid dispersion, tight junction opening, anionic drug delivery, and gas separation membranes.

3.2 Future work

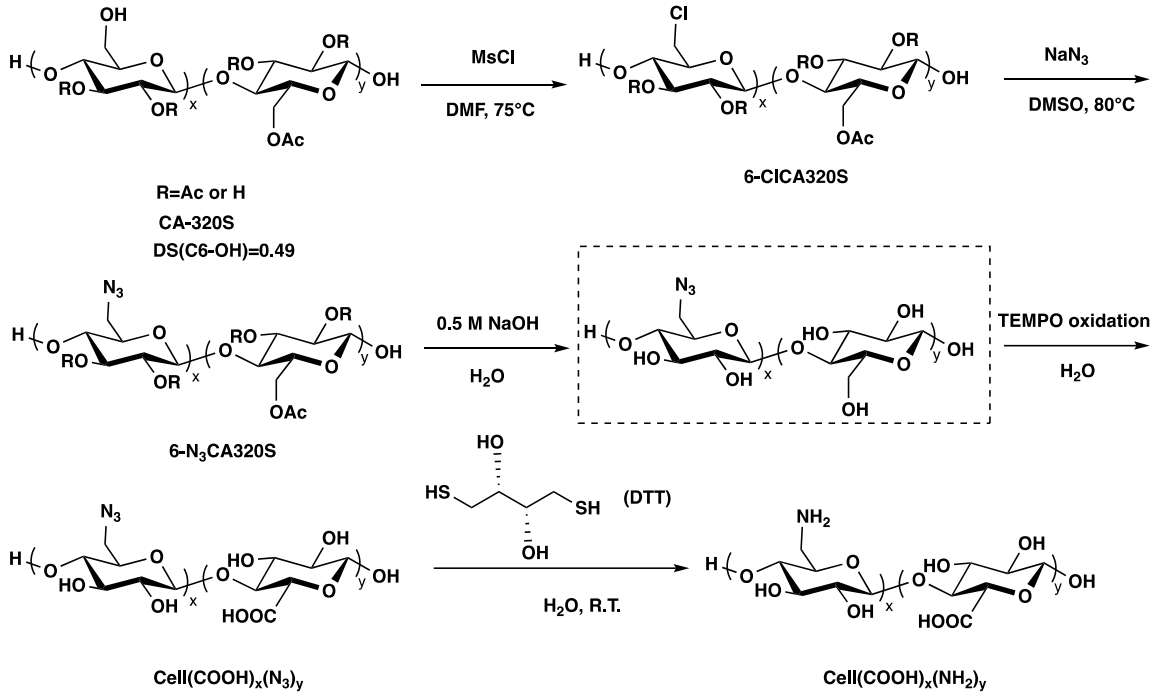
3.2.1 Synthesis of glycosaminoglycan analogues

Glycosaminoglycans (GAGs) are among the most important families of biomacromolecules, with enormous influence on physiological functions including prevention of blood clotting, development of the central nervous system, adhesion of cells to one another, signaling and recognition, joint lubrication, and innumerable other critical functions.[1] However, understanding of the structure activity relationship of GAGs is

limited by the availability of GAG analogues because of the extreme complexity of most natural GAGs (e.g., heparin, heparan sulfate, chondroitin sulfate, and dermatan sulfate), and the resulting extreme difficulty of their *de novo* synthesis. Extraction from nature and total synthesis are the most frequently used method to obtain GAGs and their analogus. Isolated GAGs may be contaminated by proteins and other polysaccharide sulfate impurities with different molecular weight and sulfate distributions, due to the presence in most tissues of structurally similar GAGs, and the affinity of GAGs for proteins (not only covalent bonding in proteoglycans, but also strong hydrogen bonding interactions as between HA and certain proteoglycans). GAG contamination is of great concern with regard to undesired toxicity and/or eliciting of immune responses by contaminants. Total synthesis of GAGs from monosaccharide building blocks is useful, but incredibly labor-intensive, expensive, and inefficient. The multistep nature of total synthesis makes it nearly impossible to synthesize GAGs with native DP (50-250). Chemical modification of polysaccharides is a practical and efficient method to obtain polysaccharide derivatives at relatively large scale. However, it has never been reported to obtain GAGs and their analogues by chemical modification of common polysaccharide. This post-mofication of common, inexpensive polysaccharides will provide an alternative novel method to obtain GAGs and their analogues. We will design a random copolymer, $Cell(COOH)_x(NH_2)_y$, as a GAG analogue. This GAG analogue will bear both carboxyl and amino groups, targeting DS of both amine and carboxyl groups at 0.5. The DS of amine and carboxyl groups of the

GAG analogue would then be identical to natural GAGs, although natural GAGs are alternating rather than random copolymers.

Other investigators have reported some methods to introduce carboxyl[2–5] or amine[6–9] groups to polysaccharide backbones. Therefore, it is possible to introduce both carboxyl groups and amino groups to polysaccharides. Employing the 6-N_3CA320S that we synthesized in Chapter 3 as a key intermediate, a possible synthetic route is proposed (Scheme 3.1). 6-N_3CA320S is prepared by regioselective chlorination and subsequent azide displacement. 6-N_3CA320S will be saponified to expose all the hydroxyl groups, followed by one-pot TEMPO-mediated oxidation of O-6, affording a water-soluble polymer, Cell$(N_3)_x(COONa)_y$. Finally, we propose to reduce azides to amines by a new, green method, using a mild reduction reagent, DTT, in water, yielding Cell$(NH_2)_x(COONa)_y$ as the first member of a new family of GAG analogues.



Scheme 3.1 Proposed synthetic route to GAG analogues

We expect this GAG analogue to exhibit promising biological activities and we can also process it into hydrogels[10] for potential drug delivery application. Furthermore, subsequent sulfation of this GAG analogue is possible, which allows us to synthesize a library of GAG analogues with different sulfation distribution patterns (including *N*-sulfated, *O*-sulfated, and *N*,*O*-sulfated) and systematically study GAG structure activity relationships.

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Appendix

Chapter 3: Regioselective chlorination of cellulose esters by methanesulfonyl chloride

Table A2.1 Degrees of Substitution (DS) of Perpropionylated CA320S.

	DS(Ac)	DS(Pr)
6-	0.51	0.49
2,3-	1.14	0.84

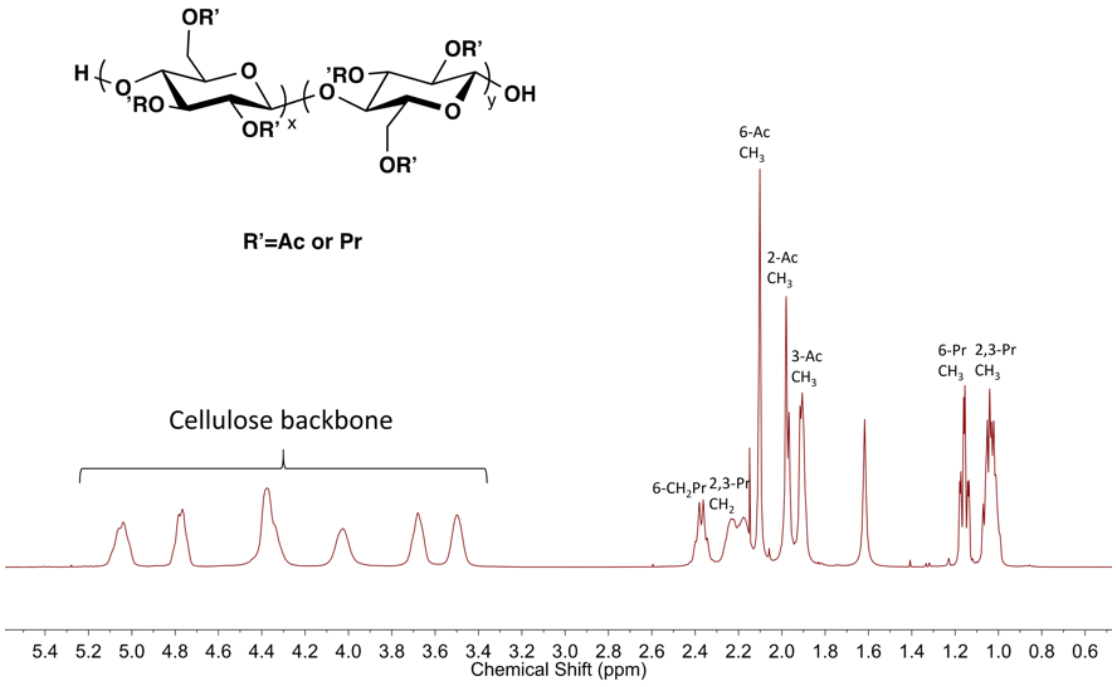

Figure A2.1 ^{1}H NMR spectrum of perpropionylated CA320S.

Table A2.2. SEC data for CA320S, 6-ClCA320S and 6-N_3CA320S

	Mn (kDa)	Mw (kDa)	PDI	DP
CA320S	30.9	47.6	1.5	131.2
6-ClCA320S	36.5	55.9	1.5	149.1
6-N_3CA320S	32.5	50.4	1.6	131.0

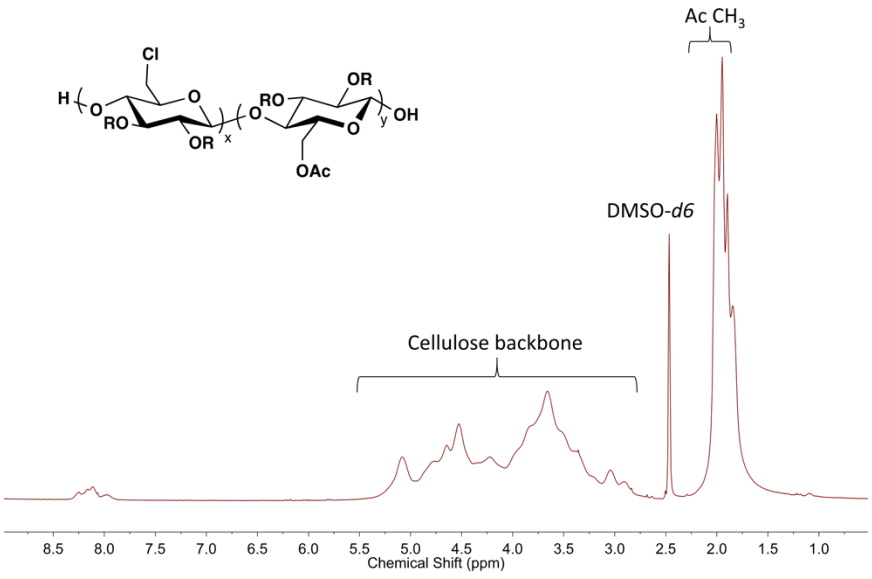

Figure A2.2 ^{1}H NMR spectrum of 6-ClCA320S.

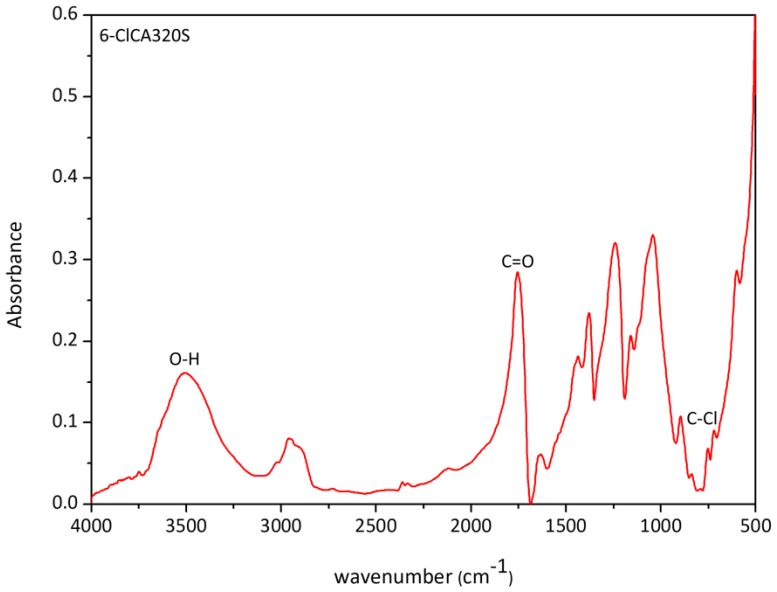

Figure A2.3 FT-IR spectrum of 6-ClCA320S.

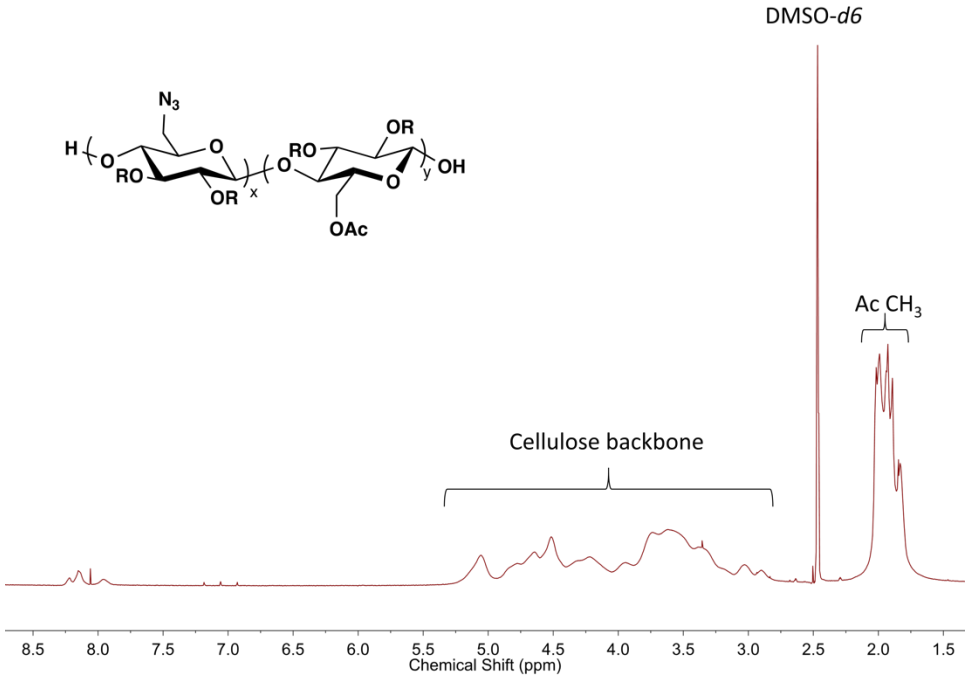

Figure A2.4 ^{1}H NMR spectrum of 6-N_3CA320S.

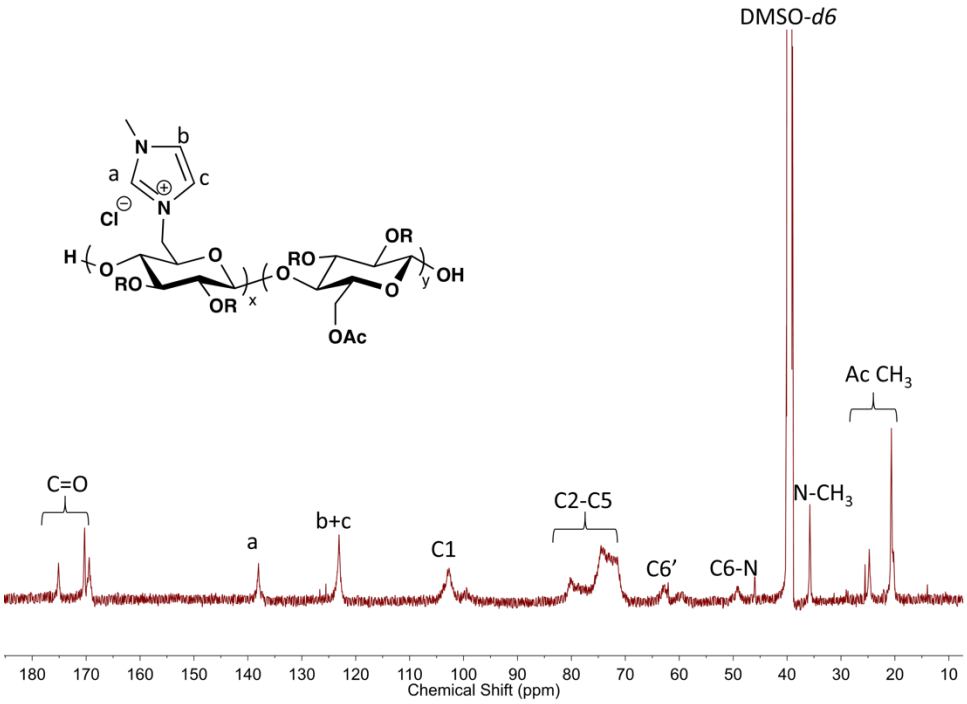

Figure A2.5 ^{13}C NMR spectrum of 6-MeIMCA320S.

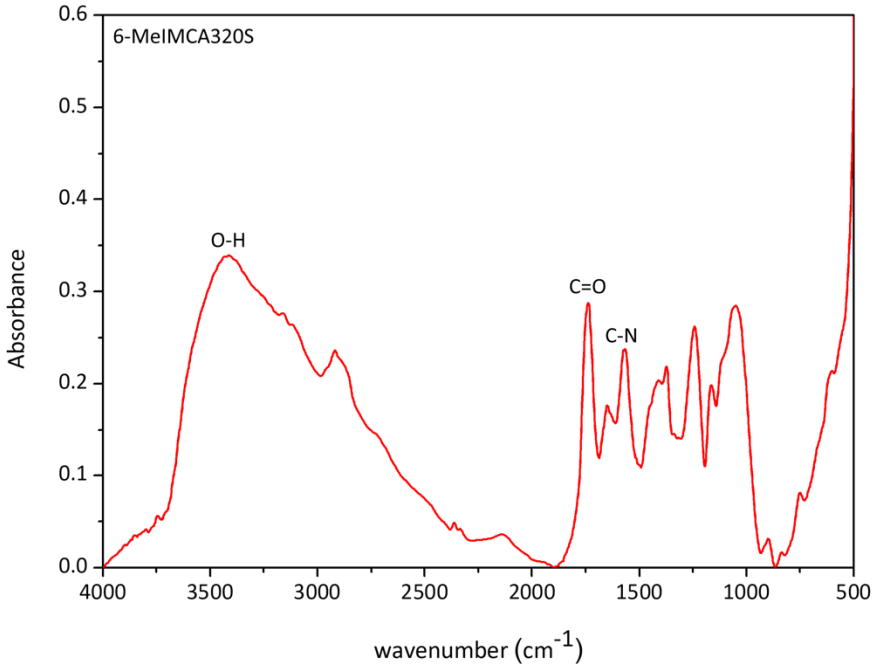

Figure A2.6 FT-IR spectrum of 6-MeIMCA320S.

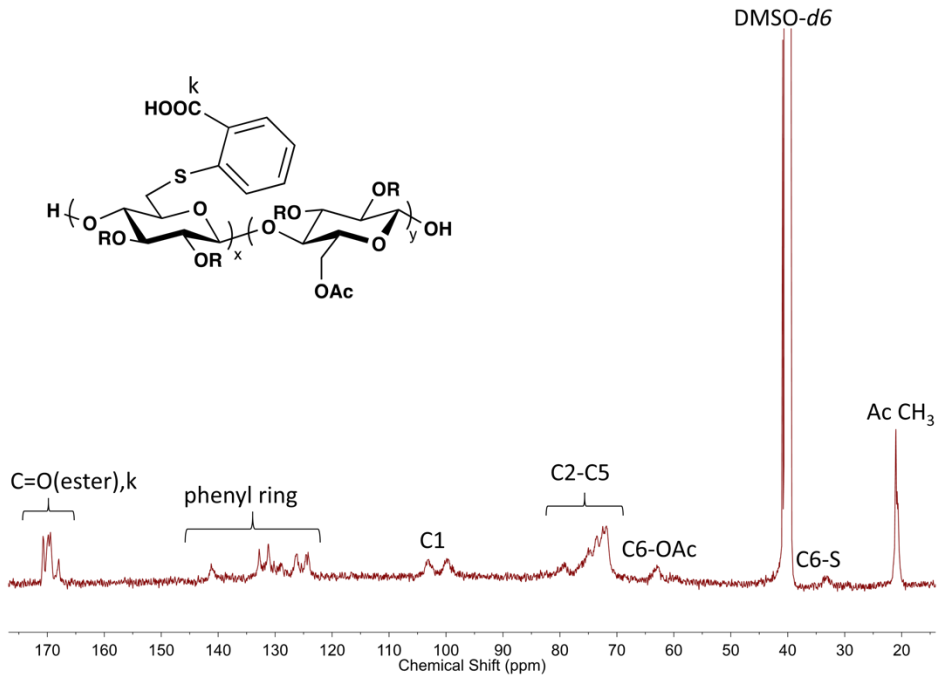

Figure A2.7 ^{13}C NMR spectrum of 6-MBACA320S.

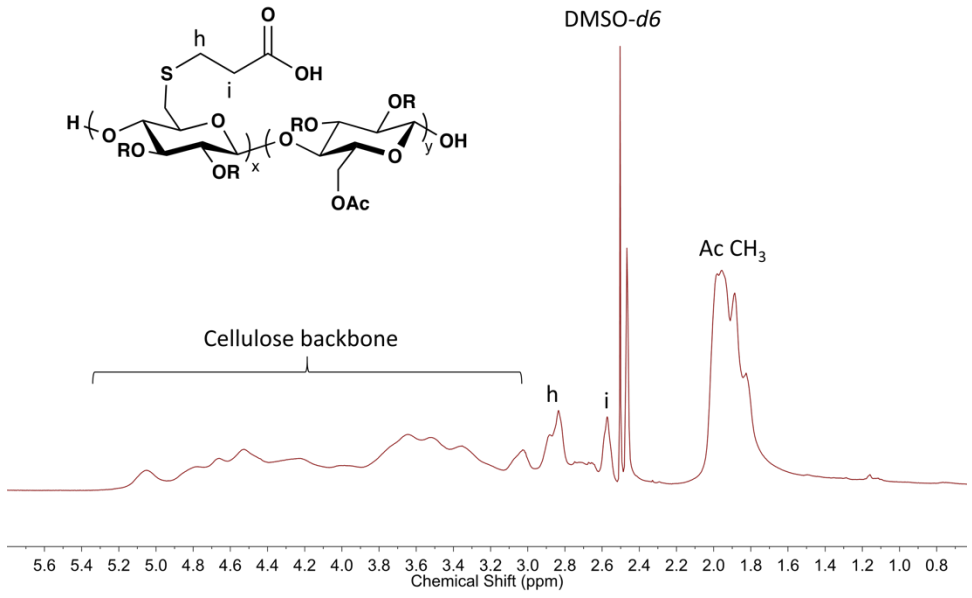

Figure A2.8 ^{1}H NMR spectrum of 6-MPACA320S.

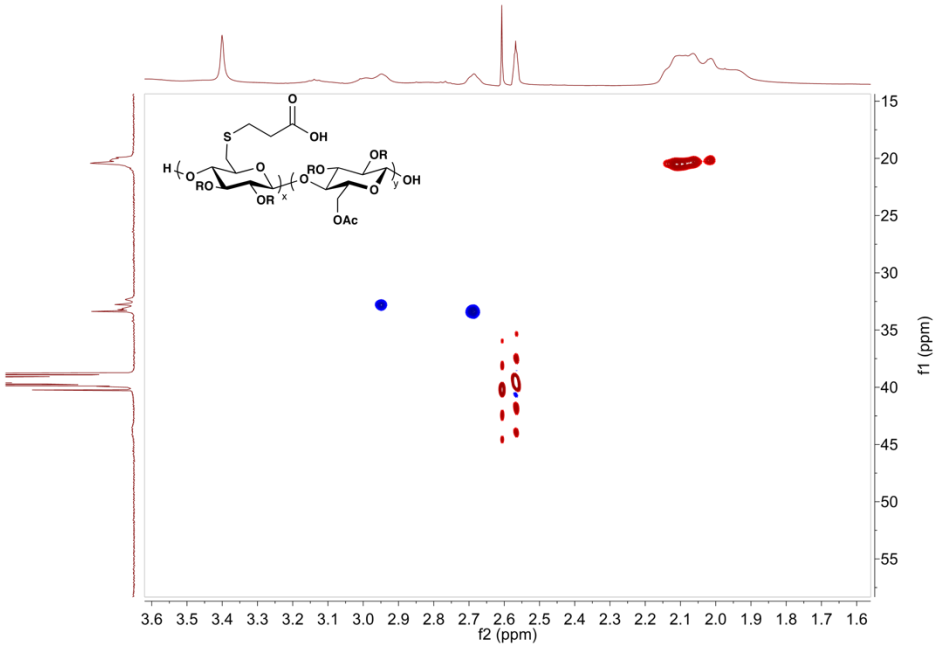

Figure A2.9 2D HSQC spectrum of 6-MPACA320S.