Regioselective Synthesis of Novel Cellulose Derivatives for Drug Delivery

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ABSTRACT

New methods were developed for the regioselective synthesis of new classes of cellulose derivatives with properties that could help improve the delivery of pharmaceutical drugs within the human body. The specific synthetic targets of this research were regioselectively carboxylated and regioselectively aminated cellulose derivatives. While different avenues to the carboxylated cellulose were ultimately explored without success, a new method for the synthesis of selectively *O*-acylated 6-amino-6-deoxy-cellulose esters was devised.

A key reaction that enabled the synthesis of the new cellulose derivatives described in this dissertation was the one-pot conversion of microcrystalline cellulose to 6-bromo-6-deoxy-cellulose esters. This reaction resulted in the highly selective displacement of the primary hydroxyl groups attached to the 6-carbon (*C*-6) on each anhydroglucose unit (AGU) in cellulose with bromide, with little or no bromination occurring at carbons 2 and 3 (*C*-2 and *C*-3). The brominated cellulose was then completely esterified by adding acetic, propionoic, or butyric acid anhydride to the reaction solution. The reaction products were readily soluble in many common organic solvents, including acetone, dimethyl sulfoxide, dimethylformamide, tetrahydrofuran, and chloroform. It was shown that the bromides could be converted to iodides under Finkelstein reaction conditions.

The presence of halides at *C*-6 allows a variety of new functional groups to be regioselectively introduced to cellulose via nucleophilic substitution. In one case, the 6-bromo-6-deoxy-cellulose esters were reacted with sodium cyanide to produce regioselectively synthesized cellulose nitriles. These compounds were synthesized with the idea that they could be converted to regioselectively carboxylated cellulose derivatives as an alternative pathway to the rhodium-catalyzed carbonyl insertion reactions also attempted in this research. However, the cellulose

nitriles were highly susceptible to alkaline degradation, and conversion to the carboxylated cellulose was not achieved.

The 6-bromo-6-deoxy-cellulose esters were also reacted with sodium azide to successfully produce 6-azido-6-deoxy-cellulose esters. The azide groups were then reduced to amines using the Staudinger reaction. This very mild and selective reaction allowed the conversion of the azides to amines in the presence of the ester groups still attached to the cellulose backbone. Such derivatives could have properties useful for a range of biomedical applications, including the delivery of anionic drugs.

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CHAPTER 1: INTRODUCTION

Cellulose is among the most ubiquitous materials in the world. As one of the main chemical constituents of plant cell walls, it can be found in abundance nearly anywhere there is life on land. One estimate has been made that cellulose is bio-synthesized on the scale of trillions of tons per year (Krässig 1993). It then comes as no surprise that such an abundant material has played a pivotal role in the human development of technology throughout history. Fibers made from cellulose have been formed into sheets to make paper or woven into textiles for millennia. More recently, chemically modified cellulose was crucial in the development of a fundamental understanding of polymer science. Cellulose nitrates mixed with camphor are credited as being among the first plastics produced artificially (Klemm, et al. 2005), and cellulose acetate was used by Hermann Staudinger in his experiments to prove that many small molecules could be covalently bonded together to form high molecular weight polymer chains (Staudinger 1920). Today, products made from cellulose, such as paper, cardboard, and cotton textiles, are widely produced on an industrial scale, and different cellulose derivatives are commonly found in food products, pharmaceutical formulations, coatings, and cosmetics (Klemm, et al. 2005). Advances in cellulose research are still being made at a rapid pace, driven in part by the wide variety of potential functional materials that can be made from the compound, as well as by the desire for new materials derived from renewable resources.

A crucial step for the continued advancement of cellulose research is the development of a better understanding of the structure/property relationships of cellulose derivatives. It is generally well known how the type of substituent and degree of substitution (DS) in cellulose derivatives affects their physical properties. However, less understood is how physical properties vary with the distribution of the substituents along the cellulose backbone. Fortunately, recent advances in cellulose research have made it possible to gain greater control over substitution patterns, particularly with respect to the regioselective derivatization of cellulose (Fox, et al. 2011). This will enable work that can help advance the understanding of structure/property relationships in cellulose derivatives. The concept of regioselectivity in cellulose chemistry will be explained in detail in the following chapter and will be a major theme of this dissertation.

A second theme of this dissertation will be the use of cellulose derivatives in pharmaceutical drug delivery. Drug delivery is the science of guiding drug molecules in the body to the necessary sites of action, in the necessary quantities for therapeutic effect, within a given timeframe, and all the while avoiding undesired and potentially toxic side effects of the drug. Cellulose derivatives already have an established record of success in oral drug delivery. For example, when properly mixed together with active drug molecules, cellulose derivatives can improve the aqueous solubility of poorly soluble drugs, control the release rate of the drug into the body, affect pH controlled drug release in the gastrointestinal system, and protect the drug from the harsh chemical environment inside the stomach (Edgar 2007; Klein 2009). However, drug delivery as a science is still maturing, and while cellulose derivatives and other polymeric carriers have already been proven extremely useful, there is still much room for improvement. Many drugs still have inadequately addressed issues with poor aqueous solubility, one of the primary factors that can prohibit a drug from entering circulation within the body, and a large fraction of new drugs under development are projected to have these same issues with poor solubility because of the current nature of the drug discovery process (Lipinski, et al. 2001). Due to their versatility and lack of toxicity, derivatives of cellulose and other polysaccharides are good candidates for helping overcome some of these obstacles in drug delivery.

The research presented in this dissertation began with the idea of developing regioselective synthetic reactions to produce new cellulose derivatives with properties that could be applied to drug delivery. It was thought that the use of regioselective techniques could allow for greater control over the material properties of the cellulose derivatives than traditional non-selective techniques. The new regioselective synthetic methods that were developed with this research will be described, along with some derivatives that were produced using these methods. It is believed that the synthetic methods advanced here could be useful for producing a number of other potentially interesting cellulose derivatives in the future.

An outline for this dissertation is as follows: **Chapter 2** will discuss some fundamental aspects of cellulose chemistry, and then it will give a detailed review of research already performed on the regioselective derivatization of cellulose. The chapter will close with a review of the use of cellulose in drug delivery applications. **Chapter 3** will then present the first synthesis and characterization of regioselectively brominated cellulose esters. These compounds will be used as a starting material for synthetic reactions in the remainder of the research

discussed in this dissertation. **Chapter 4** will discuss attempts to synthesize regioselectively carboxylated cellulose derivatives. While the reactions in this chapter were ultimately unsuccessful, the first synthesis and characterization of 6-cyano-6-deoxy-cellulose esters was achieved. **Chapter 5** will cover the synthesis of 6-amino-6-deoxy-cellulose esters via the mild and selective reduction of the azide groups in 6-azido-6-deoxy-cellulose esters. These reactions are believed to be the first demonstration of the Staudinger reduction (named after the same scientist previously mentioned that proved the existence of polymers) on polysaccharide-based substrates. A new method for the regioselective acylation of cellulose will also be presented. **Chapter 6** will discuss the conversion of the brominated cellulose esters to iodinated cellulose esters along with their use in the synthesis of new polycationic cellulose derivatives. **Chapter 7** will provide a summary of the research results in this dissertation and suggest a course for future experiments.

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CHAPTER 2: LITERATURE REVIEW

Introduction

The main focus of this review will be on the regioselective derivatization of cellulose. First, a brief background section will cover basic cellulose chemistry, with a focus on aspects that are important for cellulose derivatization. Several more thorough reviews of the physical properties and the fundamental chemistry of cellulose have been published, including those by Klemm and coworkers (Klemm, et al. 1998b, a; Klemm, et al. 2002; Klemm, et al. 2005). The second section will be a comprehensive review of the literature covering the regioselective derivatization of cellulose, with a focus on techniques used to synthesize cellulosic compounds with a highly regular repeating structure. Since one of the objectives of this research is to investigate new cellulose derivatives for drug delivery applications, the last section will discuss how cellulose and its derivatives have been used in oral drug delivery.

Cellulose Background

Sources of cellulose

Plants

Most cellulose obtained for commercial or laboratory purposes is isolated from plants. Cellulose forms structural fibers in plant cell walls that, when combined with the other plant polymers hemicellulose, pectin, and lignin, form a natural structural composite material possessing the necessary strength and rigidity to allow the plant to grow upwards towards the sun. The actual fraction of cellulose found in plant cell walls varies from source to source, but woody

plants typically contain about 40 – 50% cellulose by weight. Table 2.1, adapted from Klemm et al. (2002), gives the chemical composition of some plant materials. Cellulose may also be found in other parts of a plant outside of a cell wall, such as in the seed hairs of cotton, which are comprised of nearly pure (≈ 95%) cellulose fibers.

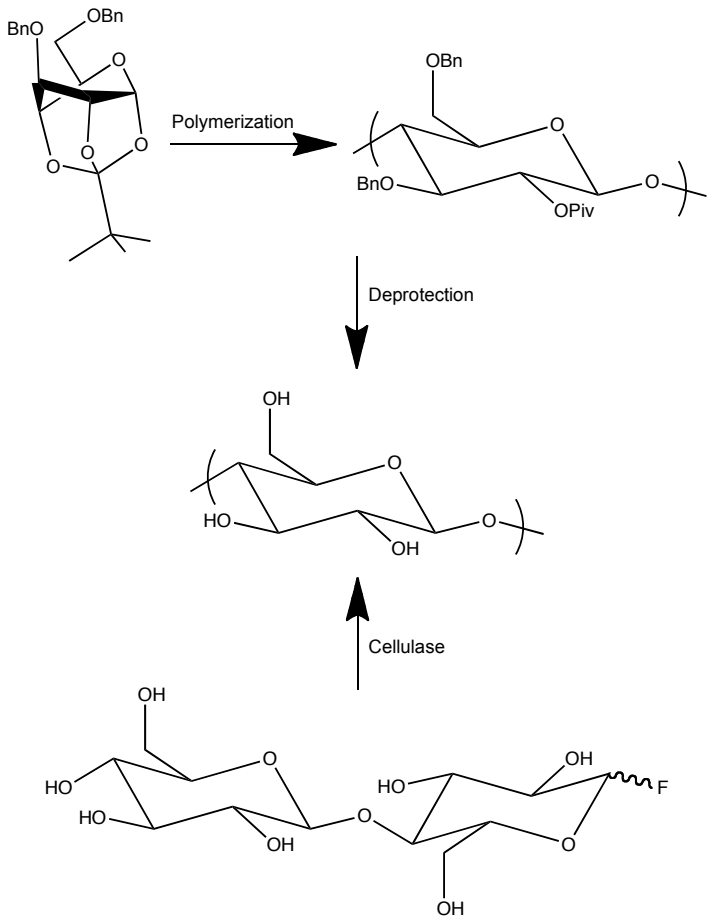
The isolation of cellulose fiber from wood is one of the largest industrial activities in the world. The two major industrial processes that digest wood pulp to isolate cellulose fiber from the other plant polymers are the sulfite process, which uses aqueous sodium sulfite at 130 – 140 °C, and the Kraft process, which uses aqueous sodium hydroxide and sodium sulfide at 170 – 180 °C (Fengel and Wegener 1989). These processes degrade and dissolve the lignin and hemicellulose present in wood, leaving the cellulose behind. The cellulose is then often bleached to further remove impurities and increase its brightness. The cellulose, not unaffected by these processes, has its polymeric structure degraded to a lower molecular weight and oxidized to a low degree, resulting in the presence of some carbonyl groups within the cellulose molecule. Both the amount of residual impurities and the final molecular weight of the isolated cellulose depend on its original plant source as well as the specific method by which it was isolated. Kraft and sulfite pulps can have degrees of polymerization (DP's) in the range of 300 to 2000. (Molecular weight can be calculated by multiplying the DP of a cellulose sample by 162 g/mol, which is the molecular weight of cellulose's monosaccharide repeating unit.) Cellulose that has been further degraded by acid or enzyme hydrolysis to produce microcrystalline cellulose has a DP between 150 and 300. Native cellulose plant or cotton fibers may have a DP approaching 12,000 (Klemm, et al. 1998a; Klemm, et al. 2002; Klemm, et al. 2005).

Table 2.1 Chemical composition of some plant materials (Klemm, et al. 2002)

	% Composition			
Source	Cellulose	Hemicellulose	Lignin	Extractives
Hardwood	43 – 47	25 – 35	16 – 24	2 – 8
Softwood	40 – 44	25 – 29	25 – 31	1 – 5
Bagasse	40	30	20	10
Corn cobs	45	35	15	5
Corn stalks	35	25	35	5
Cotton	95	2	1	0.4
Hemp	70	22	6	2
Jute	71	14	13	2
Kenaf	36	21	18	2
Ramie	76	17	1	6
Sisal	73	14	11	2
Wheat straw	30	50	15	5

Other sources

Highly crystalline cellulose, free from the plant-derived impurities, is produced by some species of bacteria (for example, *Gluconacetobacter xylinum* and *Acanthamoeba castellani*) and algae (for example, *Valonia ventricosa* and *Chaetamorpha melagonicum*). Cellulose harvested from these sources is typically only used in the laboratory and is, due to its high crystallinity, well suited for studies on the structure of the polymer (Klemm, et al. 2002).



Scheme 2.1 *In vitro* cellulose synthesis pathways

In vitro synthesis of cellulose was first achieved by the polymerization of *ß*-D-cellobiosyl fluoride with cellulase enzymes (Kobayashi, et al. 1991). The DP of the cellulose produced was reported to be about 22. The first purely artificial chemical synthesis of cellulose was performed by Nakatsubo et al. (1996) by step-wise ring-opening polymerization of 3,6-di-*O*-benzyl-*α*-D-glucopyranose 1,2,4-orthopivalate. Subsequent deprotection of the polymer yields cellulose with a DP as high as 55 (Scheme 2.1). These methods have been reviewed by Kobayashi et al. (2001) and Faijes and Planas (2007).

The structure of cellulose

Chemical structure

Cellulose is a linear homopolymer of the monosaccharide D-glucose (Figure 2.1). For identification purposes, each carbon atom in a glucose molecule can be numbered 1 through 6 (*C*-1 through *C*-6), starting by convention at the molecule's anomeric carbon. During the cellulose synthesis, each glucose molecule is linked to the next by a condensation reaction between the hemiacetal at *C*-1 of the first molecule and the hydroxyl group at *C*-4 on the adjacent molecule. Thus, since each glucose monomer loses a molecule of water in this process, the repeating structure of cellulose is comprised of *anhydro*glucose units (AGUs).

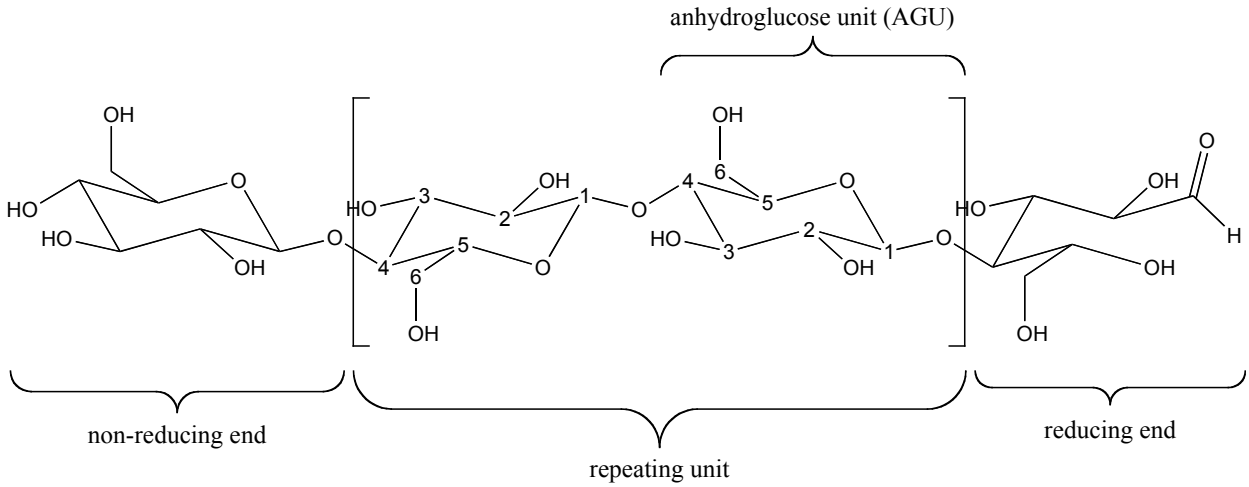


Figure 2.1 The molecular structure of cellulose, with the carbons of the repeating structure numbered 1 through 6

The acetal oxygen atom connecting the AGUs is in the equatorial, or *beta*, position, and accordingly the bond between each AGU is referred to as a *ß*-(1→4)-glycosidic bond. The pyran ring of the AGUs in cellulose has been shown by ^{1}H-NMR spectroscopy to adopt the $^{4}C_{1}$ chair conformation, with the three free hydroxyl groups attached to *C*-2, *C*-3, and *C*-6 also in the equatorial plane of the ring. Rotation about the glycosidic bonds in cellulose is restricted by hydrogen bonding (discussed in further detail below), causing each AGU to be held at a 180º angle to its neighboring AGUs. As a result, the actual repeat structure of cellulose is made up of

two consecutive AGUs, although the term degree of polymerization (DP) as it refers to cellulose is defined as the number of AGUs in a cellulose chain. The end groups on an unmodified cellulose polymer chain consist of a free hydroxyl group on *C*-4 at the non-reducing end, and a group at the reducing end that can freely undergo mutarotation between an aldehyde and a cyclic hemiacetal. The molecular weight of a cellulose sample is dependent on both its source and the method with which it was isolated, as discussed further below, though a *β*-(1→4)-linked glucan with a DP of 20 to 30 will exhibit all the properties of cellulose (Kobayashi, et al. 2001).

Hydrogen bonding and crystallinity

The supramolecular structure of cellulose plays an extremely important role in its chemical reactivity and its physical properties. Strong inter- and intramolecular hydrogen bonding between the numerous hydroxyl groups on the cellulose backbone makes cellulose relatively difficult to dissolve into a homogeneous solution, and is also the reason that cellulose does not undergo a melt transition below its thermal degradation temperature. The strength of the hydrogen bonding network is also aided by the linear, non-branching morphology of the polymer and the equatorial position of the hydroxyl groups, factors that allow the cellulose chains to pack tightly together.

The degree of crystallinity of cellulose is typically in the range of 40% to 60%, though it can cover a wide range depending on its source and the method used to isolate it. Five different allomorphic forms have been discovered for crystalline cellulose (Gilbert and Kadla 1998). Crystallites in native cellulose are known to occur as cellulose I (Figure 2.2). In this form, the cellulose chains are packed parallel to each other. Intrachain hydrogen bonding has been shown to occur between the hydroxyl groups attached to *C*-2 and *C*-6, as well as between the hydroxyl group attached to *C*-3 and the ring oxygen on the adjacent AGU. Intermolecular hydrogen bonding occurs between the hydroxyl groups at *C*-6 and *C*-3. Two different variations of the cellulose I allomorph were discovered in the 1980's by solid-state ^{13}C NMR, notated as cellulose I_α and I_β. Both forms of cellulose I can be found alongside each other in nature, though cellulose I_α is thought to be the dominant form found in samples isolated from bacteria and algae, while cellulose I_β is the more abundant form in plants. It has been shown that cellulose I_α can be irreversibly changed to cellulose I_β by annealing the former with heat (Gilbert and Kadla 1998).

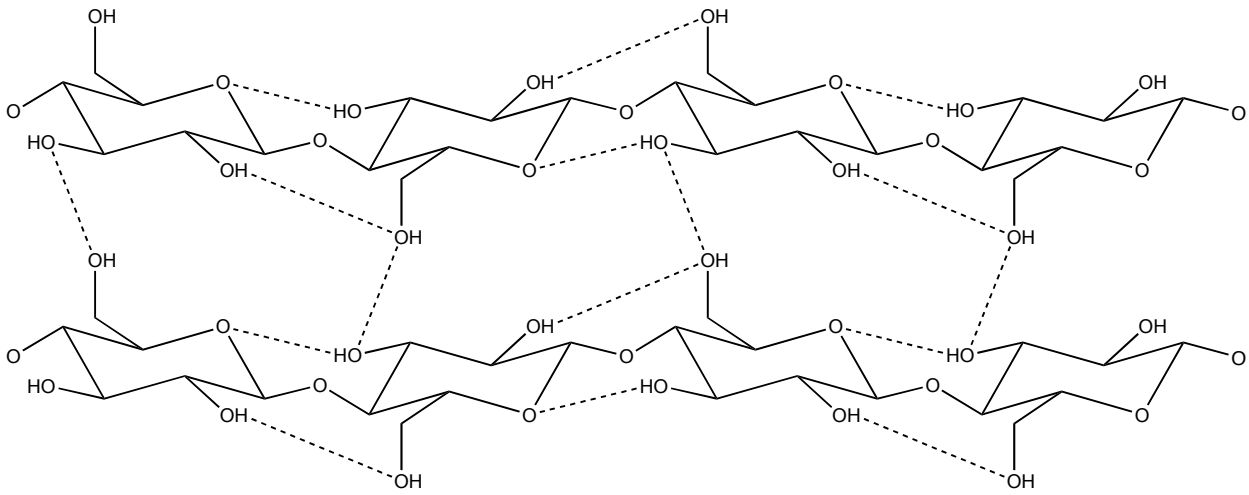


Figure 2.2 Depiction of inter- and intramolecular hydrogen bonding in a cellulose I segment, shown by the dashed lines

The cellulose II allomorph is formed irreversibly from cellulose I either by regenerating dissolved cellulose from solution or by mercerization of cellulose. Mercerization is the swelling of cellulose by soaking it in an alkali bath. From a technical standpoint, cellulose II is important because cellulose regeneration and mercerization are widely used industrial practices. In cellulose II, the polymer chains are oriented anti-parallel to each other, in contrast with cellulose I. The intramolecular hydrogen bonds in cellulose II are similar to those in cellulose I, though the intermolecular bonds are more complex (Gilbert and Kadla 1998).

Cellulose III and cellulose IV are less important from a technical point of view, though they have been produced and characterized in the laboratory. Cellulose III is formed by treating cellulose II with liquid ammonia or organic amines. Cellulose IV is formed by the treatment of cellulose III with glycerol at high temperatures (around 260 °C) (Gilbert and Kadla 1998).

Chemical modification of cellulose

The three hydroxyl groups per AGU along the cellulose backbone allow ample opportunity to synthesize new derivatives of the polymer. In fact, several important commodity materials are produced on an industrial scale by attaching ester or ether substituents at these hydroxyl sites, and numerous additional chemical substituents have been attached in the laboratory. Cellulose derivatization is often undertaken to alter the solubility and thermal

properties of the polymer by interrupting the hydrogen bonding network between the hydroxyl groups.

One important parameter that dramatically influences the physical properties of a cellulose derivative is the fraction of the hydroxyl groups that have a substituent. In cellulose chemistry, this fraction is referred to as the degree of substitution (DS), which is defined as the average number of hydroxyl groups that have been derivatized in a cellulose sample per AGU. Thus, a cellulose derivative that has undergone a complete reaction and has a substituent on every hydroxyl group has a DS equal to 3, ignoring end-group effects, while partially derivatized cellulose samples will have a DS value between 0 and 3.

There are a few factors, however, that complicate cellulose derivatization. First, due to its strong hydrogen bonding network, cellulose is insoluble in most aqueous and organic solvents typically found in a laboratory. As a result, it is typically necessary either to swell cellulose prior to a heterogeneous reaction or to choose a suitable solvent system capable of dissolving cellulose for a homogeneous reaction. Second, as a polymer comprised of acetal bonds between repeating units, cellulose is prone to chemical degradation and depolymerization under certain conditions. This must be taken into account when designing reactions, especially when a high molecular weight product is desired. Third, it has been shown that the position at which substituents are attached to cellulose and their distribution along the polysaccharide backbone can have a significant effect on the material properties of the product. However, the hydroxyl groups in cellulose all have similar reactivity, and reagents used in the traditional cellulose derivatization reactions are unable to differentiate between them to any significant extent. Thus, discovering reagents and synthetic methods that can result in control over the sites of reaction along the cellulose backbone is an important research goal in cellulose chemistry. The swelling and dissolution of cellulose along with cellulose degradation pathways will be briefly discussed in this section of the review. Reactions that can differentiate between the three hydroxyl groups on each AGU will be given a more comprehensive treatment in the following section of this review.

Cellulose swelling

Cellulose does readily swell in a number of polar liquids, including water, liquid ammonia, ethanolamine, ethanol, dimethylsulfoxide (DMSO), dimethylformamide (DMF), and dimethylacetamide (DMAc) (Klemm, et al. 1998a). These liquids act by disrupting the hydrogen

bonding in the relatively accessible amorphous regions of cellulose and allowing easier access to the crystalline regions. Stronger swelling agents can disrupt some of the hydrogen bonding in the crystalline regions as well. The extent of the swelling appears to depend on the medium's polarizability and hydrogen bonding acceptor capacity, as well as on the supramolecular structure of the cellulose sample (El Seoud, et al. 2008). In general, polar protic or aprotic organic liquids with high hydrogen bonding basicity, such as DMSO or ethanolamine, swell cellulose to a greater extent than water. Simple alcohols like methanol or ethanol tend to swell cellulose less than water.

Prior to a synthetic reaction, cellulose is often put through a swelling pretreatment step, sometimes referred to as an "activation" step, to increase the accessibility for chemical derivatizing agents to the cellulose backbone. This pretreatment step is known to significantly increase the rate and extent to which derivatizing agents can react with cellulose, thus increasing the DS in the product. Swelling is commonly accomplished with an aqueous sodium hydroxide solution, especially in preparation for etherification reactions where the presence of the base catalyzes the reaction. The effect of sodium hydroxide solutions on cellulose supramolecular structure has been extensively studied (Klemm, et al. 1998a). Swelling is known to occur rapidly, and its extent is dependent on the alkali concentration and temperature. Alkali concentrations above 12 to 15% cause cellulose crystallites to change their structure from that of cellulose I to cellulose II. Other cellulose swelling methods simply allow the cellulose to soak in the solvent medium to be used for the subsequent reaction, such as DMSO, prior to adding the other reagents (Klemm, et al. 1998a).

Dissolution

A number of solvent systems have been found with a strong enough hydrogen bond disrupting capability to effectively dissolve cellulose, though only a few are really suitable for derivatization reactions (Klemm, et al. 1998a). Dissolving cellulose allows for more control over its derivatization by eliminating the influence of its supramolecular structure. Homogeneous reactions ensure statistical distribution of the conversion sites along the whole cellulose chain since the entire cellulose sample is equally accessible to the reagents. Additionally, a higher DS can be obtained with reagents that have high steric bulk, while the same reactions under

heterogeneous conditions would result in a product with a lower DS due to lower accessibility of the cellulose backbone.

In past reviews cellulose solvents have been broadly divided into two categories: derivatizing solvents and non-derivatizing solvents (Klemm, et al. 1998a). In this sense, a derivatizing solvent dissolves cellulose by forming covalent bonds with the polymer that can then be broken upon regeneration of the cellulose. Historically, probably the most important cellulose derivatizing solvent is the combination of carbon disulfide and sodium hydroxide. This system forms a water-soluble ionic cellulose xanthogenate used in the viscose process to manufacture regenerated cellulose fiber. Non-derivatizing cellulose solvents do not require the formation of covalent bonds to dissolve cellulose. As such, solvents in this category are generally better suited for use by chemists looking to perform a range of chemical derivatization reactions on cellulose. A comprehensive review of cellulose solvents has been published by Liebert (2010). The following discussion focuses on just a few specific solvent systems important for lab scale cellulose derivatization today.

One of the most important and most widely used solvent systems in cellulose research over the past couple of decades has been the DMAc/LiCl system. By using an activation step prior to dissolution, high molecular weight cellulose samples can be completely dissolved at concentrations around 4%, while lower molecular weight samples can be dissolved at concentrations approaching 15% (Klemm, et al. 1998b). The activation step is usually accomplished by a solvent exchange procedure or by heat activation. Solvent exchange involves first swelling the cellulose in water, then filtering out the cellulose and adding DMAc while the cellulose is still wet. Often, an intermediate solvent such as methanol is used prior to exchange with DMAc. This activation method is laborious, and it is difficult to remove all the moisture from the cellulose present due to the aqueous swelling step. However, it does not lead to any degradation of the cellulose (Dupont 2003). Heat activation involves heating a slurry of cellulose suspended in DMAc to between 120 °C and 160 °C for 1 to 2 hours. At these temperatures, the vapor pressure of the DMAc is such that it can adequately penetrate the cellulose fibers, allowing the cellulose to dissolve upon addition of LiCl and cooling. Heat activation is generally the preferred procedure for swelling the cellulose prior to dissolution because it is less labor intensive and time consuming than the solvent exchange method. Since DMAc, LiCl, and cellulose are all hygroscopic, heat activation can also be used to help remove traces of water in

the mixture by distilling off some of the solvent while it is at elevated temperature, which is advantageous in preparing for water sensitive chemical reactions. However, significant drawbacks do exist to heat activation. It has been shown to result in partial depolymerization of the cellulose, due to the formation of highly reactive *N*,*N*-dimethylketeniminium ions that form from DMAc in the presence of LiCl at temperatures above 80 °C (Potthast, et al. 2003). This leads to a yellowing of the solution once the cellulose has dissolved.

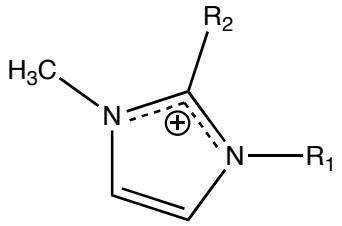
In the past decade, new solvent systems have been discovered that can dissolve cellulose without the inconvenience of an activation step. Heinze et al. (2000) reported that cellulose with a DP up to 650 can be dissolved at concentrations around 2.9% using a 10 to 20% solution of tetrabutylammonium fluoride (TBAF) in DMSO. Dissolution in this solvent takes place at room temperature and does not result in any noticeable cellulose degradation. The DMSO/TBAF solvent system has since been used for a number of cellulose derivatization reactions, including homogeneous acetylation, benzylation, and carboxymethylation. However, reactions in DMSO/TBAF can suffer from the fact that TBAF exists as a trihydrate. It is impossible to remove all traces of water from the solvent because anhydrous TBAF is thermally unstable and readily undergoes E2 elimination upon removal of water of hydration. Anhydrous DMSO/TBAF has been prepared by synthesizing the TBAF in situ from tetrabutylammonium cyanide and hexafluorobenzene, and this solvent has been used to dissolve cotton fibers with a DP of 3,743 (Köhler and Heinze 2007). However, the solution is unstable beyond 24 h.

The use of ionic liquids for dissolving cellulose is another field that has received much attention over the past decade. Ionic liquids are typically defined as salts that melt below 100 °C. Swatloski et al. (2002) first reported in 2002 that ionic liquids containing 1-alkyl-3-methylimidazolium cations, with the alkyl chain either 4 or 6 carbons long, can readily dissolve cellulose at concentrations up to 25% using only mechanical stirring and heat. Since then, many other ionic liquids have been shown to dissolve cellulose as well, and Pinkert et al. (2009) recently published a comprehensive review listing the ionic liquids that have been studied in this regard. As that paper shows, ionic liquids with imidazolium, pyridinium, phosphonium, and ammonium based cations have been investigated in conjunction with a variety of anions for their cellulose dissolving power. However, the most effective ionic liquids in terms of the amount and molecular weight ranges of cellulose they can dissolve are 1-alkyl-3-methylimidazolium or 1-alkyl-2,3-dimethylimidazolium salts. Table 2.2 summarizes the results from several studies that

examined the dissolution of cellulose in ionic liquids based on these cations. The ionic liquid 1-butyl-3-methylimidazolium chloride ($Bmim^+Cl^-$) is the most extensively studied with regard to cellulose dissolution in the literature. In their original paper on the subject, Swatloski et al. (2002) reported that cellulose with a DP around 1,000 could be dissolved in $Bmim^+Cl^-$ at a concentration of 10% by simply heating a slurry of the cellulose in the ionic liquid to 100 °C and stirring. Interestingly, careful heating of the $Bmim^+Cl^-$ with microwaves allowed cellulose solutions at concentrations of up to 25%. They also reported on the effect of the alkyl chain length bonded to the methylimidazolium cation, as well as different anions. The same cellulose was dissolved in 1-hexyl-3-methylimidazolium chloride at a concentration of 5%, while it was only sparingly soluble in 1-octyl-3-methylimidazolium chloride. Switching the chloride anion in $Bmim^+Cl^-$ with either bromide or thiocyanate resulted in maximum cellulose concentrations of 5 to 7% in solution. Heinze et al. (2005) studied the effects of the molecular weight of the cellulose on the dissolution power of ionic liquids in $Bmim^+Cl^-$. As expected, they found that increasing the molecular weight of the cellulose makes it more difficult to dissolve in high concentrations. Barthel and Heinze (2006) studied the ionic liquids 1-ethyl-3-methylimidazolium chloride ($Emim^+Cl^-$), 1-butyl-2,3-dimethylimidazolium chloride ($Bdmim^+Cl^-$), and 1-allyl-2,3-dimethylimidazolium bromide ($Admim^+Br^-$), and found that they could dissolve cellulose at 12%, 9%, and 12% concentrations, respectively.

Table 2.2 Solubility of cellulose in different imidazolium ionic liquids

Cation structure:



R_1	R_2	Anion	Cellulose DP	Temperature (°C)	Cellulose concentration (wt.%)	Reference
Ethyl	H	Cl^-	286	90	12	Barthel and Heinze 2006
Allyl	H	Cl^-	650	80	14.5	Zhang, et al. 2005
Butyl	H	Cl^-	286	83	18	Heinze, et al. 2005
Butyl	H	Cl^-	593	83	13	Heinze, et al. 2005
Butyl	H	Cl^-	1198	83	10	Heinze, et al. 2005
Butyl	H	Cl^-	≈ 1,000	100	10	Swatloski, et al. 2002
Butyl	H	Cl^-	≈ 1,000	Microwave heating	25	Swatloski, et al. 2002
Ethyl	H	Ac^-	586	90 – 130	13.5	Kosan, et al. 2008
Butyl	H	Br^-	≈ 1,000	Microwave heating	5 - 7	Swatloski, et al. 2002
Butyl	H	SCN^-	≈ 1,000	Microwave heating	5 - 7	Swatloski, et al. 2002
Butyl	H	Ac^-	586	90 – 130	13.2	Kosan, et al. 2008
Hexyl	H	Cl^-	≈ 1,000	100	5	Swatloski, et al. 2002
Butyl	Methyl	Cl^-	377	80	9	Barthel and Heinze 2006
Allyl	Methyl	Br^-	286	80	12	Barthel and Heinze 2006

Homogeneous reactions with cellulose dissolved in DMAc/LiCl, DMSO/TBAF, and various ionic liquids have all been used to synthesize a range of cellulose derivatives, many of which are inaccessible through heterogeneous reactions. The use of both DMAc/LiCl and DMSO/TBAF as solvents for reactions to produce cellulose derivatives with bulky ester substituents was demonstrated by Liebert and Heinze (2005) and by Heinze et al. (2007). Granström et al. (2008) used the ionic liquid 1-allyl-3-methylimidazolium as a solvent to

produce different bulky ester derivatives of cellulose. One issue common to each of these homogeneous media for derivatizing cellulose, however, is the fact that they are all hygroscopic. This becomes important in esterification or other reactions where the resulting substituents can be hydrolyzed, lowering the DS of the product. In DMAc/LiCl or ionic liquids, the effect of moisture can be minimized by the use of pre-dried reagents. In DMSO/TBAF, however, the removal of moisture is difficult because of the presence of water in TBAF as discussed above. As expected, the concentration of TBAF in esterification reactions has a substantial effect on the DS of the final product (Ass, et al. 2004). Both DMSO/TBAF and ionic liquids do have the advantage of not requiring an activation step prior to dissolution. The activation required for cellulose dissolution in DMAc/LiCl will likely result in the presence of additional moisture or some degree of degradation of the cellulose, depending on whether solvent exchange or heat activation is used. Ionic liquids are often considered green solvents because of their extremely low vapor pressure and the potential to recycle them after reactions (Pinkert, et al. 2009). Because of these factors and the ease with which they dissolve cellulose, their utility in cellulose chemistry is likely to continue to grow.

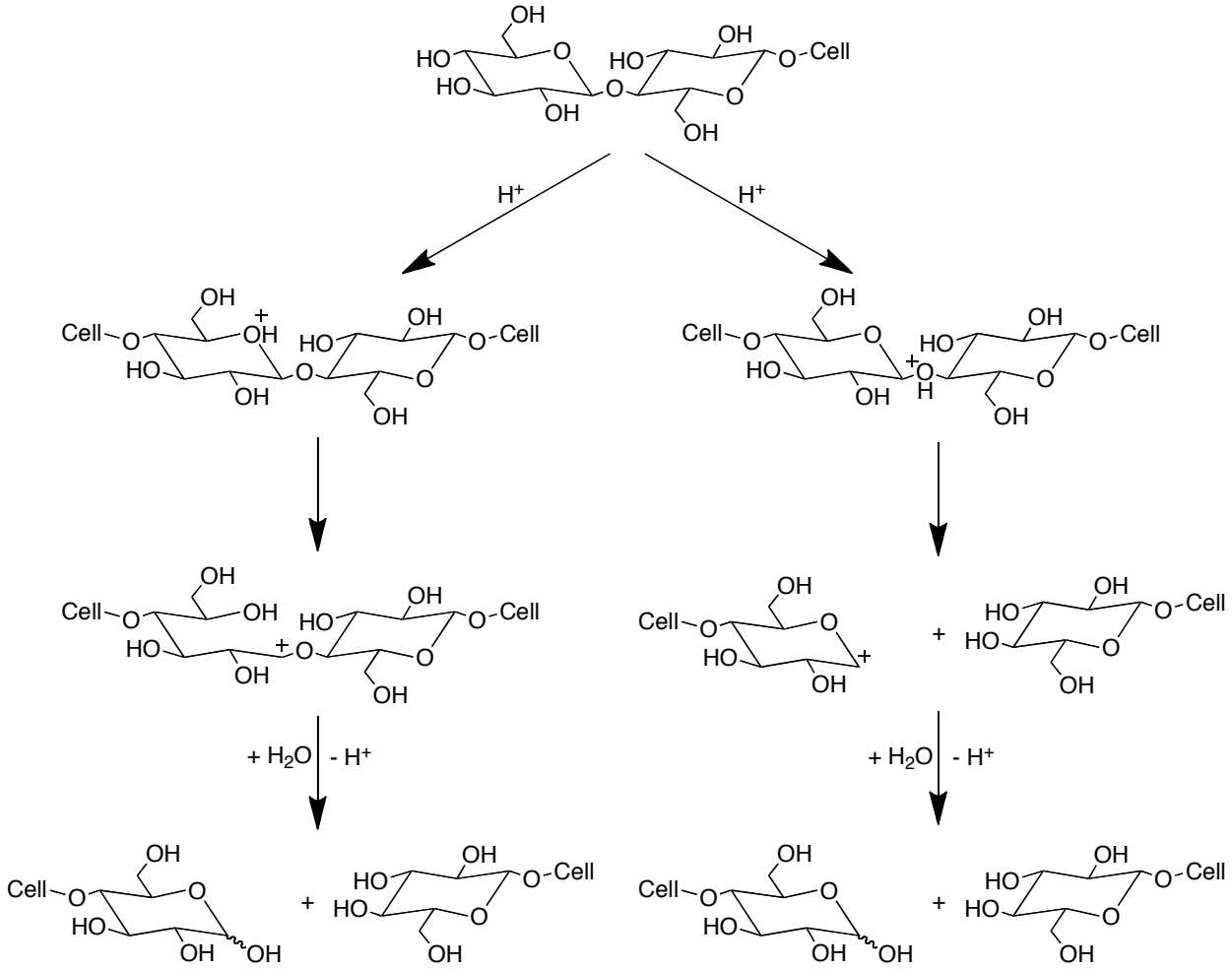
Degradation

The primary routes for chemical degradation of cellulose are by acid or enzyme hydrolysis, alkaline peeling, and oxidation. As is the case with other cellulose reactions, the rate and extent to which these degradation pathways proceed is limited by the tight crystalline packing of the cellulose chains, hindering access to reaction sites. This phenomenon is particularly evident in the hydrolysis of cellulose by dilute mineral acids. Under these conditions, the molecular weight of the cellulose will initially decrease rapidly as the acid attacks the amorphous regions of the polymer. As the reaction continues, however, the rate of hydrolysis decreases and eventually falls to zero, at which point the cellulose is said to have reached the level-off DP. This occurs because the remaining cellulose fibers are highly crystalline, and the acid is unable to penetrate the crystalline matrix. The value of the level-off DP is largely determined by the source of the cellulose and any pretreatment steps it has undergone. Native celluloses with large crystallites have a level-off DP around 300, whereas cellulose that has been treated to increase its amorphous content may have a level-off DP below 100 (Klemm, et al. 1998a). Cellulose can be completely hydrolyzed by higher concentrations of mineral acids at

elevated temperature. Specifically, a 72% sulfuric acid solution is often used for this purpose. The sulfuric acid is thought to be able to swell the intracrystalline regions of the cellulose fiber enough to gain access to the glycosidic bonds within, and is therefore able to degrade cellulose all the way to its monomeric components.

The mechanism for acid hydrolysis is shown in Scheme 2.2. First, the glycosidic bond is protonated either at the ring oxygen or the bridging oxygen. In either case, a carbocation is subsequently formed at the carbon 1 position, which is then susceptible to attack by water. The acid proton is regenerated as two new cellulose chain ends form (Klemm, et al. 1998a; Gilbert and Kadla 1998).

Cellulase enzymes produced by certain strains of fungi and bacteria are able to hydrolyze cellulose at ambient or slightly elevated temperatures in a pH range of 4 to 9. Cellulases are actually a mixture of three different types of enzymes: endoglucanases, exoglucanases, and β-D-glucosidase. Endoglucanases can attack cellulose anywhere within the chain and cause the most rapid reduction in molecular weight of the three cellulases. Exoglucanases are only able to cleave off glucose units from the ends of a cellulose chain. The third cellulase, β-D-glucosidase, attacks cellobiose to break it down into two glucose molecules. Again, the rate of cellulose degradation is dependent on the crystallinity of the sample since the large cellulase proteins cannot easily penetrate tightly packed crystallites. The size and distribution of substituents on cellulose do have an impact on the ability of cellulases to degrade the polymer. In general, it seems cellulases are still able to degrade cellulose derivatives that contain small substituents (e.g. methyl ether groups) or have low DS values, while higher DS values and larger substituents hinder the ability of cellulases to recognize the cellulose molecule. There is some evidence that cellulases require three unsubstituted AGUs in a row to be able to attach to the cellulose backbone and hydrolyze it (Klemm, et al. 1998a).

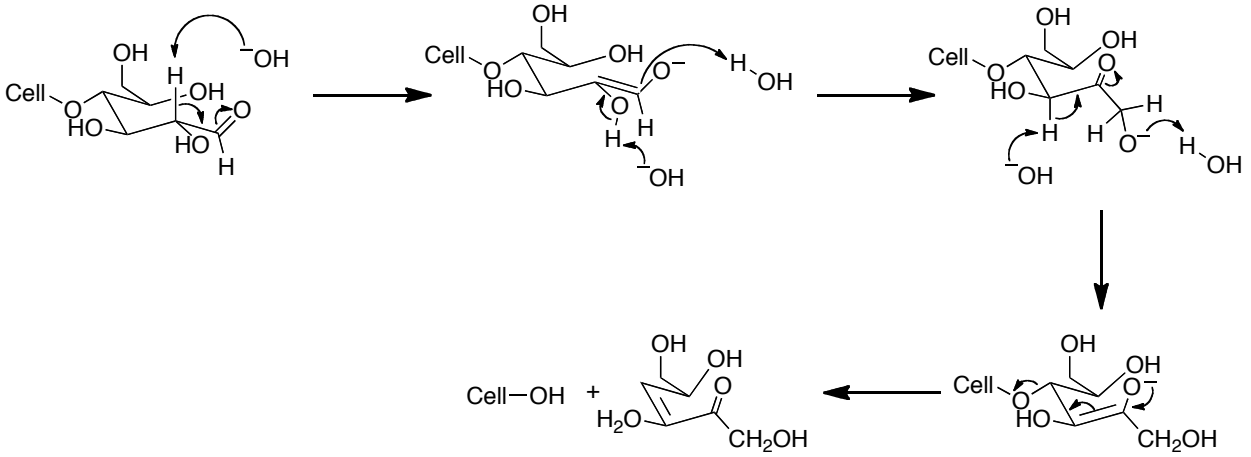


Scheme 2.2 Mechanism for acid hydrolysis of cellulose

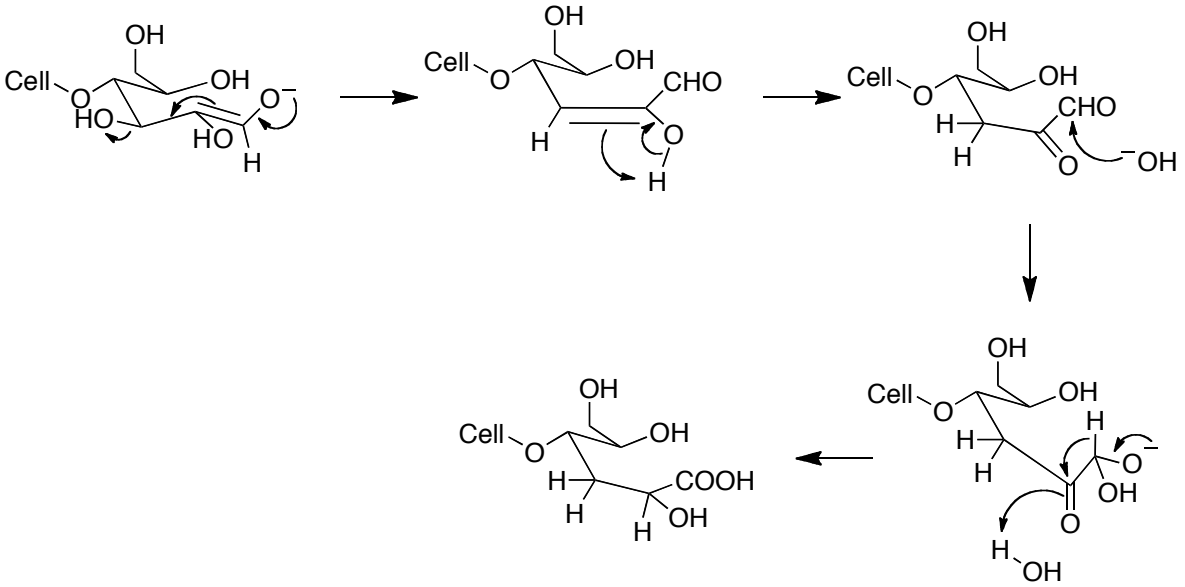
Alkaline degradation of cellulose is of particular importance to the Kraft pulping process, which is the most common industrial method for isolating cellulose fiber from wood. The primary mechanism for alkaline degradation occurs by a "peeling" reaction that cleaves the AGU at the reducing end of a cellulose chain. Since this only cleaves one AGU at a time, this reaction does not rapidly reduce the DP of cellulose. This reaction proceeds by a series of elimination reactions on protons adjacent to carbonyl groups, beginning with the reducing end aldehyde. The mechanism for alkaline peeling of cellulose is shown in Scheme 2.3. This reaction can be halted by a competing reaction in which the first enolate intermediate instead undergoes a β-hydroxy elimination and is converted into a carboxylic acid group that is stable to alkaline attack (Scheme 2.4). At elevated temperatures of around 150 °C, the glycosidic bonds in cellulose

become susceptible to alkaline hydrolysis. This type of alkaline degradation can cause a significant reduction in molecular weight of the cellulose (Klemm, et al. 1998a; Gilbert and Kadla 1998).

Oxidative degradation of cellulose is somewhat more complex than acid, alkaline, or enzymatic degradation in that it does not necessarily follow a well-defined mechanism, and it is highly dependent on the conditions present. It is an important process, however, as most wood pulps typically contain some small degree of oxidized functionalities due to bleaching steps in the pulping process. Cellulose is rather stable to atmospheric oxygen except under highly alkaline conditions (pH > 14). Oxidation can take place at any of the hydroxyl groups on cellulose, forming aldehyde groups at C-6 or ketone groups at C-2 and C-3. Aldehyde groups can also form at C-2 or C-3 with ring cleavage. When any of these carbonyls form, the cellulose is prone to β proton elimination by a base, further exacerbating the degradation process. Under the proper conditions, such as by treatment with N_2O_4 or N_2O_3, the carbonyls can be further oxidized to carboxylic acids. Oxidation by potassium chromate in sulfuric acid is known to degrade cellulose all the way to carbon dioxide and water.



Scheme 2.3 Cellulose alkaline peeling mechanism, beginning at the reducing end of the chain



Scheme 2.4 Competing reaction to alkaline hydrolysis of cellulose that halts depolymerization

Regioselective Derivatization of Cellulose

Regioselective derivatization of cellulose may have different definitions depending on the context in which it is used. Generally, it refers to reactions that result in chemical substituents attached at one or two of the three chemically discrete hydroxyl groups on each AGU along the cellulose backbone, while reactions at the remaining hydroxyl sites generally do not occur. The result is a highly regular repeating structure of the cellulosic polymer, with each AGU containing the same substituents at the same positions. This is usually achieved by the use of bulky reagents that favor reactions with the primary hydroxyl group attached to *C*-6 over reactions with the secondary hydroxyl groups attached to *C*-2 and *C*-3. An alternative definition for regioselective derivatization of cellulose could be used to describe reactions that only occur along relatively short, discrete sections of the polymer backbone, resulting in a block co-polymer type of structure. This section of the review will focus on the first definition of regioselectivity – differentiation between the three hydroxyl groups on each AGU. To narrow the focus further, it will mainly consider reactions that are regarded as highly selective for certain hydroxyl sites.

As research into the regioselective synthesis of cellulose derivatives has progressed, it has become increasingly apparent that by controlling the sites at which chemical substituents are attached to cellulose, one can control the physical properties of the resulting compounds. In one

example, Kondo (1997) compared the solubilities of commercial methylcellulose derivatives to regioselectively synthesized methylcelluloses produced in the laboratory. As shown in Table 2.3, reproduced from Kondo's paper, 6-*O*-methylcellulose has much higher solubility in organic solvents than commercial methylcelluloses with a comparable DS. While this table says that 2,3-di-*O*-methylcellulose is insoluble in water, Kern et al. (2000) produced the same cellulose derivative with even better regioselective control over the reaction, and found that the product was in fact water-soluble. This finding was used to illustrate how, in certain cases, small differences in substituent distribution along the cellulose backbone can result in large differences in solubility. The physical properties of regioselective cellulose derivatives will be discussed further in this section of the review.

Table 2.3 Solubility of commercial and regioselective methylcelluloses, adapted from Kondo (1997)

	Commercial Methylcellulose				
Solvent	DS 0.1-1.1	DS 1.4-2.0	DS 2.4-2.8	23MC DS 2.0	6MC DS 1.0
Water	△	○	×	×*	×
Aq. Alkali (pH 9.5)	○	△	×	△	△
Aq. Acid (pH 5.5)	○	○	△	○	○△
Acetone	×	×	×	×	○
Methanol	×	×	×	△	○
THF	×	×	○	×	○
Chloroform	×	×	○	△	○
DMSO	○△	○	○△	○	○
DMAc	○△	○	○△	○	○

23MC = 2,3-di-*O*-methylcellulose; 6MC = 6-*O*-methylcellulose

○ = soluble; △ = swelling; × = insoluble; ○△ = partially soluble

* perfectly regioselective 2,3-di-*O*-methylcellulose was later found to be water soluble (Kern, et al. 2000)

A few different strategies exist for the regioselective synthesis of cellulose derivatives. The oldest and most widely practiced strategy is the use of protecting groups. Such groups will react with cellulose, blocking the hydroxyl sites they react with from further derivatization. Then the remaining free hydroxyl groups can be subjected to reactions such as etherification or esterification. Following that, the protecting groups can be removed, regenerating the original hydroxyl groups at those specific sites. This strategy has been proven very effective with cellulose, though it can often be labor intensive. Another strategy is the *de novo* synthesis of regioselective cellulose derivatives, or synthesis of the derivatives from monomeric compounds. A third strategy involves the introduction of substituents, specifically at the C-6 position, that can act as good leaving groups for further substitution reactions. This can be referred to as "activation" of C-6. If the activating groups are stable under the appropriate conditions, they can also act as blocking groups during reactions at the C-2 and C-3 positions. These three strategies will be discussed in detail in this section.

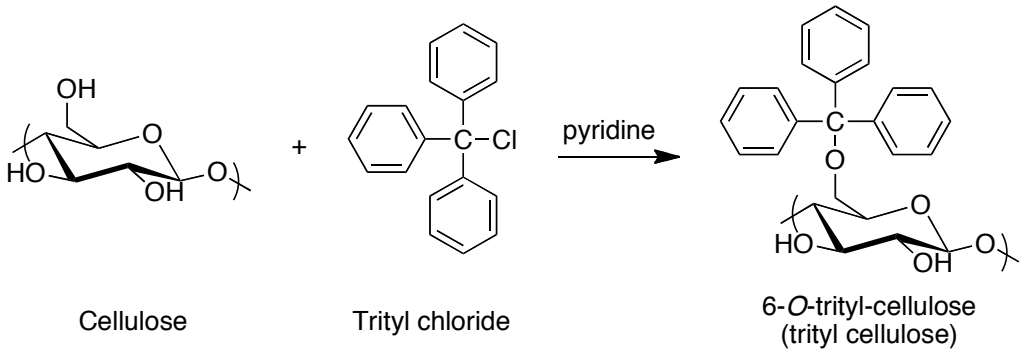
Protecting group strategies

Tritylation

The reaction of cellulose with triphenylchloromethane, hereafter referred to as trityl chloride, is one of the oldest and most effective protecting group strategies for synthesizing regioselectively modified cellulose derivatives (Scheme 2.5). It has long been known that, due to steric demands, trityl chloride reacts preferentially with the primary hydroxyl group attached to *C*-6 on the cellulose backbone rather than with either of the secondary hydroxyl groups attached to *C*-2 and *C*-3 (Helferich and Köster 1924; Hearon, et al. 1943; Honeyman 1947; Hall and Horne 1973). Furthermore, the trityl group can be quantitatively removed by acid hydrolysis after derivatization of the secondary hydroxyl groups with acid stable functionalities (alkyl ethers, for example) to regenerate the free hydroxyl group at *C*-6. This allows a variety of different cellulose derivatives to be synthesized where the hydroxyl substituents at the *C*-2 and *C*-3 positions are different from the substituent at the *C*-6 position.

Tritylation of crystalline cellulose fails to produce a readily soluble material. Thus, as is often the case throughout cellulose chemistry, the cellulose must be activated in some manner to disrupt its crystallinity prior to tritylation to obtain a soluble product. A historically common method to activate cellulose for tritylation is the complete deacetylation of cellulose acetate in

aqueous ammonium hydroxide (Hearon, et al. 1943). Mercerized cellulose is also sometimes used (Kern, et al. 2000). With these methods, the activated cellulose is then transferred to dry pyridine to form a slurry that becomes homogeneous as the reaction progresses. More recently, however, the cellulose solvent DMAc/LiCl is typically used as the reaction medium as it is a less time and labor intensive procedure (Takahashi, et al. 1986; Erler, et al. 1992). Tritylation of cellulose has also been performed homogeneously on cellulose dissolved in the ionic liquids 1-butyl-3-methylimidazolium chloride (Erdmenger, et al. 2007) and 1-allyl-3-methylimidazolium chloride (Granström, et al. 2009). The DS of the trityl group can be in the range of 0.93 to 1.07, depending on the reaction temperature, the molar ratios of the reactants, and the duration of the reaction. Approximately 90% of the substituents are found at the *C*-6 position, while a small proportion of the secondary hydroxyls at *C*-2 and *C*-3 are also tritylated (Hearon, et al. 1943; Kondo and Gray 1991).



Scheme 2.5 Preparation of 6-*O*-trityl cellulose

Heinze et al. (1994) and Gómez et al. (1996) reported that the use of methoxy substituted trityl chlorides significantly increased the rate of hydroxyl substitution on cellulose. A DS of 0.96 could be achieved in only 4 h at 70 °C using *p*-monomethoxytriphenylmethyl chloride as the tritylation reagent, whereas use of the unsubstituted trityl chloride required over 24 h to achieve the same DS under similar reaction conditions. The selectivity for the *C*-6 position of the *p*-monomethoxytriphenylmethyl chloride was found to be similar to that of the unsubstituted trityl chloride. The increase in the reaction rate was attributed to the stabilization of the triarylmethyl cation reactive intermediate by the electron donating methoxy group. Additionally,

the removal of methoxy substituted trityl groups from *C*-6 by acid treatment only requires about 5.5 h, while complete removal of unsubstituted trityl groups requires 100 h. This is particularly beneficial since exposure of the cellulose to acid during detritylation can result in reduction of the molecular weight of the polymer (Hearon, et al. 1943). In fact, Kern et al. (2000) reported that the molecular weight of 2,3-di-*O*-methyl cellulose ethers synthesized using microcrystalline cellulose as the starting material had not significantly decreased after acid hydrolysis of monomethoxy trityl groups from C-6. Additionally, the solubility in organic solvents of cellulose samples derivatized with alkoxy substituted trityl groups can be modified by increasing the alkoxy chain length (Ifuku, et al. 2004).

Tritylation chemistry has made possible many structure/property relationship studies on cellulose derivatives substituted at the *C*-2 and *C*-3 positions. Among the first of these compounds synthesized and characterized were 2,3-di-*O*-methyl- and 2,3-di-*O*-ethyl-cellulose (Kondo and Gray 1991). Kern et al. (2000) studied the behavior of 2,3-di-*O*-methyl-cellulose in aqueous solutions. They reported that subtle variations in the DS and distribution of the methyl substituents can have dramatic effects on the phase separation behavior of the cellulose derivative. Over-methylated cellulose samples ($DS_{meth} > 2.0$) with a significant number of AGUs containing three methyl substituents would phase separate upon heating of the aqueous solution. This observation lends support to the hypothesis that phase separation in methylcellulose solutions is due to the aggregation of these tri-substituted AGUs. However, phase separation from aqueous solution at elevated temperatures was also observed in under-methylated cellulose samples ($DS_{meth} < 2.0$) containing many mono-methylated AGUs but no tri-methylated anhydroglucose units, suggesting that mono-methylated AGUs are also prone to aggregation. The phase separation occurred at a higher temperature in the under-methylated samples compared to the over-methylated samples. No phase separation was observed in the pure 2,3-di-*O*-methyl-cellulose ($DS_{meth} = 2.0$) aqueous solutions, and it was concluded that the di-methylated anhydroglucose units do not aggregate upon heating. Petzold-Welke et al. (2010) found that the water solubility of cellulose methylated at the *C*-2 and *C*-3 positions with a DS around 0.90 was dependent on the frequency of occurrence of di-substituted AGUs along the cellulose backbone. Samples containing more di-substituted AGUs were water soluble, while samples containing more mono-substituted AGUs tended to be water insoluble.

Synthesis of 6-*O*-alkyl cellulose ethers by tritylation chemistry is somewhat more problematic than the synthesis of 2,3-di-*O*-alkyl-celluloses. After the trityl cellulose has been made, appropriate protecting groups must be chosen for the *C*-2 and *C*-3 positions. These protecting groups must be stable to acid hydrolysis during the detritylation step, and then they must be stable to alkaline conditions during the alkylation of *C*-6. Finally, they must still be easily removed after the alkylation step. Kondo (1993) provided a solution to this problem by choosing allyl protecting groups for *C*-2 and *C*-3. After detritylation, the 2,3-*O*-allyl-cellulose was isomerized to 2,3-di-*O*-(1-propenyl)-cellulose using potassium *t*-butoxide. Then, after alkylation at *C*-6, the 1-propenyl groups were removed by acid hydrolysis. It was reported that this method provided better results than using benzyl groups as the protecting groups at *C*-2 and *C*-3.

Inter- and intramolecular hydrogen bonding in regioselectively methylated cellulose samples was studied by FTIR and solid state ^{13}C NMR (Kondo 1994). The analyses indicated that intermolecular hydrogen bonding was largely absent in 6-*O*-methylcellulose, though it was prominent in 2,3-di-*O*-methylcelluloses. This suggests that a free primary hydroxyl group is necessary for strong intermolecular hydrogen bonding in methylcelluloses. These studies were extended to the investigations of hydrogen bonding within blends of regioselectively methylated cellulose with poly(ethylene oxide) or poly(vinyl alcohol) (Kondo, et al. 1994), as well as the analysis of hydrogen bonding in aqueous solutions of regioselectively methylated cellulose (Kondo 1997). It was found that the specific interactions of the methylcellulose with the synthetic polymers are highly dependent on the sites of substitution on the cellulose backbone. The 6-*O*-methylcellulose had high solubility in a variety of organic solvents due to the lack of intermolecular hydrogen bonding between cellulose chains, whereas 2,3-di-*O*-methylcellulose was only soluble in polar aprotic solvents.

Tritylation chemistry has been used to regioselectively synthesize analogues to other important commercial cellulose ethers. Hydroxyethyl and hydroxypropyl derivatives of trityl cellulose were heterogeneously synthesized in a mixture of isopropanol and water (Schaller and Heinze 2005). With the aid of ionic and non-ionic surfactants, a molar degree of substitution of 2.0 was obtained for both the hydroxyethyl and hydroxypropyl derivatives. NMR analysis of the products showed that the ethylene oxide and propylene oxide reagents reacted preferentially with the free secondary hydroxyl groups on the cellulose backbone, rather than with any free hydroxyl

groups on the hydroxyalkyl side chains. The authors did not propose any explanation for this preference in their reaction system, even though traditionally produced hydroxylethyl and hydroxylpropyl cellulose derivatives contain any elongated oxyethylene and oxypropylene side chains. After detritylation, the 2,3-*O*-hydroxylethyl- and 2,3-*O*-hydroxypropyl-cellulose were soluble in water at a molar degree of substitution of only 0.3 and 0.8, respectively. For comparison, conventionally synthesized hydroxypropyl cellulose derivatives require a molar degree of substitution of 4.0 for water solubility. Regioselectively substituted 2,3-*O*-carboxymethylcellulose was synthesized (Heinze, et al. 1994; Heinze, et al. 1999) by reacting trityl cellulose with sodium monochloroacetate followed by detritylation with hydrogen chloride in methanol. The highest DS of the carboxymethyl groups obtained was 1.91. It was reported that a DS of at least 0.6 was required for the polymer to be water soluble, compared to a DS of 0.4 for conventionally synthesized carboxymethylcellulose. However, Liu et al. (1997) reported that, using the same synthetic procedures as the previous papers, a DS of only 0.3 was required for water solubility.

Tritylation chemistry has also facilitated the study of structure-property relationships of regioselectively synthesized cellulose esters. To synthesize these compounds, trityl cellulose was esterified at the secondary hydroxyl groups by reaction with an appropriate carboxylic acid anhydride. The trityl protecting group was then removed by hydrogen bromide in a mixture of acetic acid and chloroform, and the regenerated primary hydroxyl group was esterified with a different carboxylic acid anhydride than before to produce the desired compound. ^{1}H NMR shifts for cellulose esters were assigned using 6-*O*-acetyl-2,3-di-*O*-propanoyl-cellulose and 6-*O*-propanoyl-2,3-di-*O*-acetyl-cellulose synthesized via trityl cellulose intermediates (Iwata, et al. 1992). Single crystals of 2,3-di-*O*-acetyl-6-*O*-propionyl cellulose, 6-*O*-acetyl-2,3-di-*O*-propionyl cellulose, 2,3-di-*O*-acetyl-6-*O*-butyryl cellulose, and 6-*O*-acetyl-2,3-di-*O*-butyryl cellulose were prepared from solutions in dibenzyl ether and *n*-tetradecane (Iwata, et al. 1994). The crystal structures of both 6-*O*-acetyl-2,3-di-*O*-propanoyl-cellulose and 6-*O*-propanoyl-2,3-di-*O*-acetyl-cellulose were determined by x-ray and electron diffraction analysis (Iwata, et al. 1996a, b) and the samples were further examined by atomic force microscopy (Iwata, et al. 1997a) and DSC (Iwata, et al. 1997b). Regioselectively acetylated trityl cellulose was also used to investigate the solution behavior of cellulose esters in polar solvents (Tsunashima and Hattori 2000; Tsunashima, et al. 2001). It was observed that the position of the ester substituents has a large

influence on the cellulose chain conformations and associations in solution. Kasuya et al. (2000) investigated 6-*O*-acetyl-2,3-di-*O*-benzoyl-cellulose and 2,3-di-*O*-acetyl-6-*O*-benzoyl-cellulose as stationary phases for chiral separations and found that the location of the specific substituents significantly affected chiral discrimination.

Cellulose derivatives with unconventional side groups synthesized via tritylation chemistry have been studied to determine their physical properties for use in a few advanced applications. Several papers have investigated the liquid crystalline properties of regioselectively substituted cellulose derivatives in organic solutions. Harkness and Gray (1990; 1991) found that 6-*O*-trityl-2,3-di-*O*-ethyl-cellulose, 6-*O*-trityl-2,3-di-*O*-benzyl-cellulose, and *6-O-α*-(l-naphthylmethyl)-2,3-di-*O*-pentyl-cellulose formed chiral nematic phases in several different organic solvents, and that the optical properties could be controlled by varying the structure and DS of the hydroxyl substituents. Cellulose derivatives with carbanilate substituents (Aust, et al. 1997; Derleth and Zugenmaier 1997) and poly(ethylene oxide) substituents (Yue and Cowie 2002) synthesized from trityl cellulose have also been studied for their chiroptic properties. 6-*O*- and 2,3-di-*O*-octadecyl-cellulose were successfully synthesized, cast into Langmuir-Blodgett films, and then studied by atomic force microscopy and x-ray diffractometry (Kasai, et al. 2005). Cellulose modified with carbazole side groups was investigated for its potential use in organic light emitting diode applications, and it was discovered that the placement of the carbazole groups at the *C*-2 and *C*-3 positions versus the *C*-6 position had an effect on the electronic properties of the polymer (Karakawa, et al. 2007). The thermal properties of poly(ethylene glycol) grafted cellulose were studied for their potential usefulness in phase change materials for energy storage (Li, et al. 2008). Kondo et al. (2009) synthesized cellulose-2,3-dicinnamate, cellulose-6-monoacetate-2,3-dicinnamate, and cellulose 2,3-diacetate-6-monocinnamate using trityl protected cellulose, introducing the cinnamate group to impart photosensitive and electroconductive properties to the polymer.

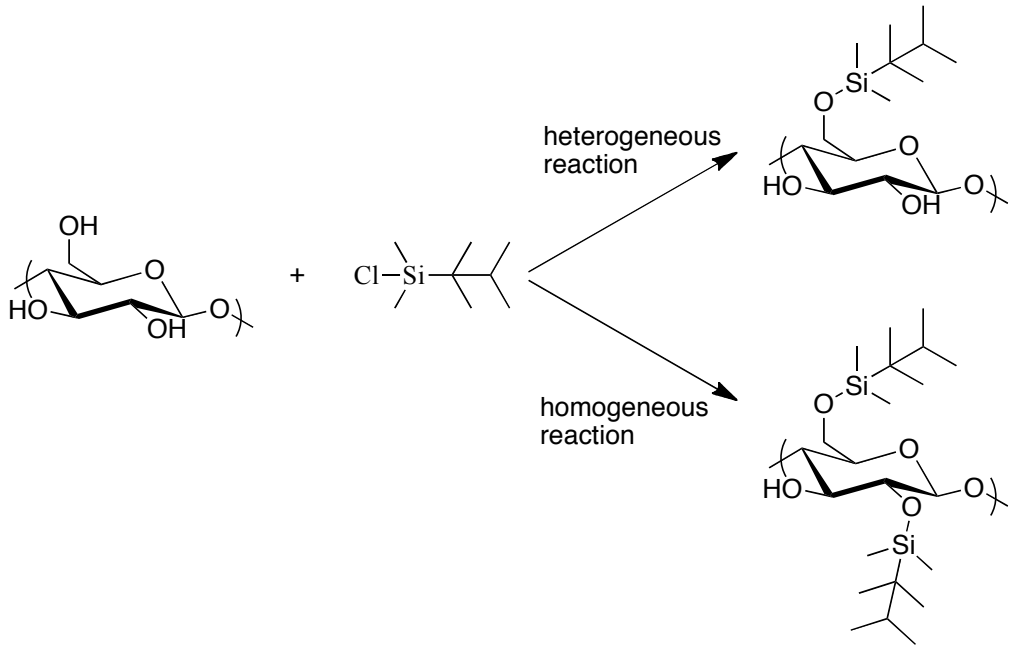
Regioselective silylation

Silylation with thexyldimethylchlorosilane (TDMS-Cl) has proven to be another effective protecting group strategy for regioselective synthesis of cellulose derivatives, and one that is perhaps more versatile than tritylation since the specific sites that are protected can be controlled by the dispersity of the cellulose in the reaction mixture (Scheme 2.6). Under heterogeneous

conditions with swollen cellulose, TDMS-Cl has been shown to react exclusively at the C-6 position forming TDMS ethers. Homogeneous reactions with dissolved cellulose and TDMS-Cl, on the other hand, are known to protect both the *C*-2 and *C*-6 hydroxyl groups. The first reported thexyldimethylsilation of cellulose was performed in DMF saturated with ammonia at -15 °C, resulting in 6-*O*-thexyldimethylsilylcellulose with a DS of 0.99 (Klemm and Stein 1995). The selectivity for the C-6 hydroxyl group was shown by NMR analysis of the permethylated samples. Subsequent heterogeneous reactions of cellulose with TDMS-Cl were run at -25 °C in ammonia saturated N-methylpyrrolidinone, and the regioselectivity was once again confirmed by HPLC and COSY NMR analysis (Koschella, et al. 1997; Koschella and Klemm 1997; Petzold, et al. 2003). Homogeneous thexyldimethylsilation reactions were first run on cellulose dissolved in DMAc/LiCl with pyridine as a base and catalyst, resulting in products with DS as high as 1.90 (Koschella and Klemm 1997). The DS and distribution of the TDMS ethers along the cellulose backbone for products produced under both homogeneous and heterogeneous reactions were confirmed by HPLC analysis of samples that were permethylated and then acid hydrolyzed. Imidazole was subsequently found to be a more effective base than pyridine for homogeneous derivatization of cellulose with TDMS-Cl, allowing the synthesis of 2,6-di-*O*-thexyldimethylsilyl cellulose with a DS of 2.0 (Koschella, et al. 2001).

Silyl protecting groups can be completely removed by treatment with tetrabutylammonium fluoride, opening up a relatively simple pathway to the synthesis of cellulose ethers substituted exclusively at the *C*-3 position. Table 2.4 lists all the 3-*O*-substituted cellulose ethers whose synthesis has been described in the literature to date, as well as their reported solubility in various solvents. A series of 3-*O*-alkyl cellulose ethers (methyl, ethyl, *n*-propyl, *n*-butyl, *n*-pentyl, isopentyl, dodecyl) has been synthesized by treatment of 2,6-di-*O*-thexyldimethylsilyl cellulose with the appropriate alkyl iodide or alkyl bromide, followed by deprotection of the 2 and 6 hydroxyl groups (Koschella, et al. 2001; Petzold, et al. 2004; Koschella, et al. 2006; Yin, et al. 2009; Heinze, et al. 2010). One interesting finding within this series is that 3-*O*-ethyl and 3-*O*-*n*-propyl cellulose were both soluble in water, though they would phase separate at temperatures above 58.5 °C and 15 °C, respectively. All the other 3-*O*-alkyl ethers were not water soluble. The hydrogen-bonding structure in 3-*O*-methyl cellulose was studied by Kondo et al. (2008). 3-*O*-Allyl cellulose was synthesized by the reaction of 2,6-di-*O*-thexyldimethylsilyl cellulose with allyl chloride followed by desilylation (Koschella, et al. 2001).

This 3-allyl derivative was later used to produce 3-*O*-hydroxypropyl cellulose with a molar substitution of 1.0 by hydroboration of the allyl double bond using 9-borabicyclo[3.3.1]nonane and subsequent alkaline oxidation with hydrogen peroxide (Schumann, et al. 2009). 3-*O*-Hydroxyethyl cellulose with a molar degree of substitution of 1.0 has also been synthesized via 2,6-di-*O*-thexyldimethylsilyl cellulose (Fenn and Heinze 2009). Both 3-*O*-hydroxyethyl cellulose and 3-*O*-hydroxypropyl cellulose were found to be water soluble. As a gateway to producing regioselective cellulose derivatives with dendritic substituents, 3-*O*-propargyl cellulose was synthesized (Fenn, et al. 2009). The terminal triple bond of the propargyl group could then undergo a copper-catalyzed Huisgen reaction to access the dendronized cellulose product. Cellulose derivatives with ethylene glycol chains of varying lengths selectively attached at the 3-hydroxyl group were synthesized with a DS of about 0.5 (Bar-Nir and Kadla 2009). These derivatives were of interest to Kadla et al. (2007) for producing cellulosic materials with a honeycomb-like structure.



Scheme 2.6 Heterogeneous and homogeneous reaction products for thexyldimethylsilyation of cellulose

The first synthesis of 2,6-di-*O*-methyl cellulose from natural cellulose was achieved using 3-*O*-allyl cellulose as an intermediate (Kamitakahara, et al. 2008). The 3-*O*-allyl cellulose was methylated at the *C*-2 and *C*-6 hydroxyl groups, and then the allyl group was removed by reaction with palladium chloride. The resulting 2,6-di-*O*-methyl cellulose was insoluble in water and common organic solvents, contrasting with the isomeric, perfectly regular 2,3-di-*O*-methyl cellulose prepared via tritylation that is soluble in water (Kern, et al. 2000).

Table 2.4 Solubility of 3-*O*-substituted cellulose ethers with free -OH groups at *C*-2 and *C*-6

3-O-Substituent[1]	Solubility	Reference
methyl	insoluble in water, DMSO, DMAc	Koschella et al. 2001
ethyl	soluble in water below 58.5 °C, DMSO, DMAc, DMF	Koschella et al. 2006
n-propyl	soluble in water below 15 °C, DMF, DMSO	Heinze et al. 2010
n-butyl[2]	insoluble in water	Yin et al. 2009
n-pentyl	soluble in MeOH, EtOH, THF, DMSO, DMF, NMP, DMAc	Petzold et al. 2004
isopentyl	soluble in MeOH, EtOH, dioxane, THF, DMSO, DMF, NMP, DMA	Petzold et al. 2004
allyl	soluble in DMSO	Koschella et al. 2001
hydroxyethyl	soluble in water	Fenn and Heinze 2009
hydroxypropyl	soluble in water, DMSO, DMAc, DMF	Schumann et al. 2009
2-methoxyethyl	soluble in water, DMSO, DMAc, NMP	Heinze and Koschella 2008
propargyl	soluble in DMSO	Fenn et al. 2009
ethylene glycol[3]	insoluble in common organic solvents	Bar-Nir and Kadla 2009
dodecyl	soluble in THF	Petzold et al. 2004

DMSO = dimethyl sulfoxide; DMAc = N,N-dimethylacetamide; DMF = N,N-dimethylformamide; NMP = N-methylpyrrolidinone; THF = tetrahydrofuran; MeOH = methanol; EtOH = ethanol

[1] DS ≈ 1.0 except when otherwise noted

[2] DS = 0.8

[3] DS = 0.5

Silylation of cellulose with tert-butyldimethylchlorosilane has also been studied for its regioselective properties in cellulose chemistry. However, it has been shown to be somewhat less selective for the *C*-2 and *C*-6 hydroxyl groups than TDMS-Cl, as some 3,6-di-*O* functionalized AGUs were also detected after derivatization. Additionally, at high reaction temperatures, some tri-functionalized AGUs were formed (Heinze, et al. 2008).

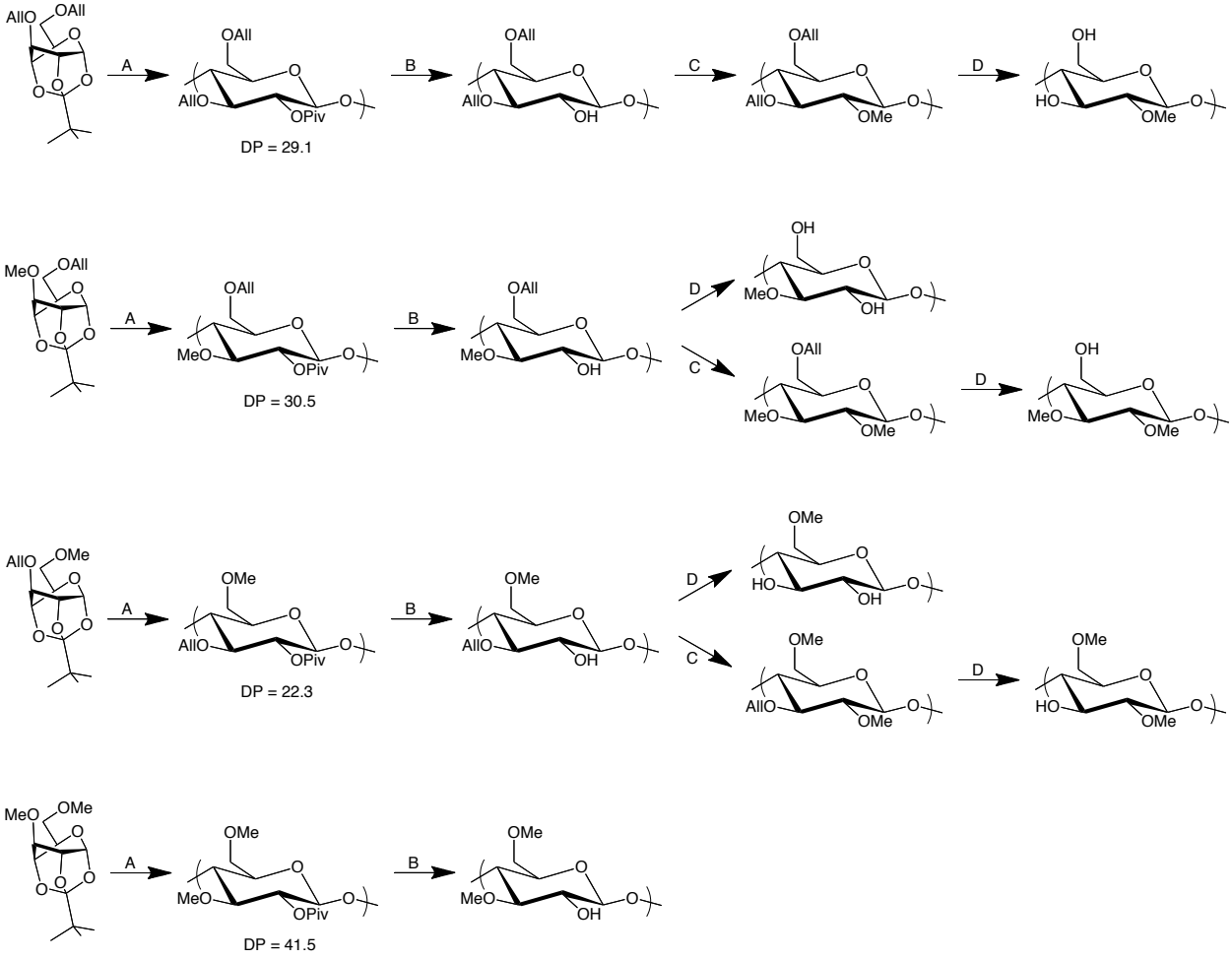
De novo synthesis of regioselectively substituted cellulose derivatives

As discussed previously, *in vitro* synthesis of cellulose has been achieved by both enzymatic polymerization of cellobiosyl fluorides using cellulases, and by step-wise ring-opening polymerization of glucose orthoesters (Scheme 2.1). The discovery of these synthetic pathways led to efforts to produce cellulose derivatives with very well-defined structures starting from monomeric compounds. Cellulose derivatives produced using these methodologies have the most well-defined substitution patterns since it is possible to completely control the structure of the monomers, and in many instances the stereochemistry of the anomeric linkages along the polymer chain. However, these methods generally are quite labor intensive and produce lower molecular weight derivatives than other regioselective cellulose derivatization pathways.

The Kobayashi group are pioneers in the enzyme-catalyzed synthesis of polysaccharides from activated monomers, reporting the first synthesis of cellulose from *β*-D-cellobiosyl fluoride using a cellulase catalyst (Kobayashi, et al. 1991). The first synthesis of a cellulose derivative by such methods used cellulase to polymerize 6-*O*-methyl-*β*-cellobiosyl fluoride, resulting in a low molecular weight 6-*O*-methyl cellulose with the methyl group present on every second AGU (Okamoto, et al. 1997). The DP of the oligomer was reported to be only 14.

Greater success at producing relatively higher molecular weight cellulose ethers has been realized over the past decade using cationic ring-opening polymerization procedures. Scheme 2.7 depicts the synthetic pathways starting from glucose orthopivalate ester monomers used to obtain the six mono- and di-substituted methyl cellulose ethers (2-*O*-methyl cellulose, 3-*O*-methyl cellulose, 6-*O*-methyl cellulose, 2,3-di-*O*-methyl cellulose, 2,6-di-*O*-methyl cellulose, and 3,6-di-*O*-methyl cellulose). These compounds were synthesized by Karakawa et al. (2002b) and then acetylated to obtain their ^{1}H and ^{13}C NMR chemical shifts. The molecular weight of the methyl cellulose ethers or their respective acetate esters were not measured directly, though the DP's of their polymeric precursors were reported (Karakawa, et al. 2002b; Karakawa, et al. 2002a).

These DP values, ranging from 22.3 to 41.5, are included in Scheme 2.7. The three regioselectively mono-substituted methyl celluloses (2-*O*-methyl-cellulose, 3-*O*-methyl-cellulose, and 6-*O*-methyl-cellulose) were further characterized by Karrasch et al. (2009) by solid state ^{13}C NMR.



Scheme 2.7 Cationic ring-opening polymerization pathways for synthesis of regioselectively modified mono- and di-substituted methyl cellulose ethers (Karakawa, et al. 2002b). All = allyl; Piv = pivaloyl; Me = methyl. Reaction conditions: A = 5 mol % $BF_3 \cdot Et_2O/CH_2Cl_2$, -30 °C, 20 h; B = NaOMe/THF:MeOH (4/1, v/v), reflux, overnight; C = CH_3I/NaOH/DMF, rt, 3 days; D = $PdCl_2$/MeOH:$CHCl_3$ (1/1, v/v), 60 °C, 4 h.

Depending on the target compound, synthesizing regioselective cellulose ethers using protecting group strategies generally requires fewer steps and results in a higher molecular

weight product. To illustrate the differences between using protecting groups and *de novo* synthesis for synthesizing regioselective cellulose derivatives, Kamitakahara et al. (2008) prepared 2,6-di-*O*-methyl-3-*O*-acetyl-cellulose via both the ring-opening polymerization of 3-*O*-benzyl-6-*O*-pivaloyl-α-D-glucopyranose 1,2,4-orthopivalate and from microcrystalline cellulose using thexyldimethylsilylation. The *de novo* pathway and the protecting group pathway each required five synthetic steps to get to the product, beginning with the glucose orthopivalate and microcrystalline cellulose, respectively. However, the microcrystalline cellulose is readily available commercially, while 3-*O*-benzyl-6-*O*-pivaloyl-α-D-glucopyranose 1,2,4-orthopivalate must be synthesized in the lab. Since synthesis of the glucose orthopivalate monomer is an involved procedure by itself, the protecting group strategy for producing 2,6-di-*O*-methyl-3-*O*-acetyl-cellulose can be considered less time and labor intensive. The ^{1}H and ^{13}C NMR spectra for the products of both synthetic pathways were nearly identical, indicating that both pathways resulted in similar chemical structures. The primary difference in the products was in their molecular weights. The product made via the thexyldimethylsilyl protecting group had a DP of about 64.6 and a polydispersity index of 8.56, while the product made via ring-opening polymerization had a DP of about 43.9 and a polydispersity index of 1.59.

Cellulose alkyl ethers with either methyl or ethyl substituents at *C*-6 (total alkyl DS = 1.0) were synthesized to determine the effect of the different alkyl groups on thermal and solubility properties (Kamitakahara, et al. 2009a). It was found that the addition of ethyl groups greatly enhances the water solubility of the compound. An aqueous solution of 6-*O*-ethyl-cellulose with no methyl substituents phase separated above 70 ºC, whereas the addition of 10% methyl groups caused the compound to remain in solution even at elevated temperatures. A similar study was performed on 2,6-di-*O*-alkyl cellulose with methyl and ethyl substituents to help elucidate the combined effects of the substituent type and position on the properties of the cellulose derivatives (Kamitakahara, et al. 2008). Table 2.5 was recreated from that paper and shows the effects of the methyl and ethyl substituents and their position on solubility. It appeared that the ethyl substituent at *C*-2 had a greater effect on increasing solubility than at *C*-6. Another study of the three regioselectively mono-ethylated cellulose ethers (2-*O*-ethyl-cellulose, 3-*O*-ethyl-cellulose, and 6-*O*-ethyl-cellulose) showed that all three were soluble in water (Kamitakahara, et al. 2010). The 2-*O*-ethyl-cellulose was water-soluble over the entire temperature range tested, though a DP of only 11 was achieved for the compound. The 3-*O*-

ethyl-cellulose, with a DP of 49, phase separated from aqueous solution at 40 ºC. Three 6-*O*-ethyl-cellulose samples were synthesized with a DP of 13, 60, and 36. The low molecular weight sample remained in aqueous solution throughout the experimental temperature range, while the higher molecular weight samples phase separated around 70 ºC. The sample with a DP of 36 exhibited thermally reversible gelation at 70 ºC, while the other two 6-*O*-ethyl-cellulose samples showed no such behavior.

Table 2.5 Solubilities of 2,6-*O*-alkyl-celluloses (Kamitakahara, et al. 2009b)

Solvent	2E6E	2E6M	6E2M
H_2O	–	–	–
MeOH	+	–	–
EtOH	+	–	–
Acetone	+	–	–
$CHCl_3$	++	++	+
MeOH/CH_2Cl_2 (1/4, v/v)	++	++	+

Solubility was evaluated at a concentration of 5 wt%

++, clear solution; +, cloudy solution; –, precipitation

2E6E = 2,6-di-*O*-ethyl-cellulose; 2E6M = 2-*O*-ethyl-6-*O*-methyl-cellulose; 6E2M = 6-*O*-ethyl-2-*O*-methyl-cellulose

To further clarify the structure-property relationships of specifically modified methyl celluloses, a series of diblock co-oligomers of tri-*O*-methylated and unmodified cello-oligosaccharides were synthesized using ring-opening polymerization (Kamitakahara, et al. 2006, 2007). Only low molecular weight oligomers were synthesized, likely due to the laboriousness of the procedure. The di-block co-oligomers were found to have good water solubility and high surface activity.

Besides simple linear alkyl chain ether derivatives of cellulose, regioselectively carboxymethylated cellulose has also been synthesized by ring-opening polymerization pathways. All three mono-carboxymethylated cellulose compounds (2-*O*-carboxymethyl-cellulose, 3-*O*-carboxymethyl -cellulose, and 6-*O*- carboxymethyl –cellulose) were synthesized by Nishio et al.

(2005). Little physical property characterization of these compounds has been reported, though all were soluble in water.

C-6 activating groups

Tosylation

The synthesis of sulfonic acid esters of cellulose, such as methanesulfonate and benzenesulfonate derivatives, has long been used in synthetic cellulose chemistry, but the reaction with *p*-toluenesulfonic (tosyl) chloride to form tosyl esters of cellulose has been the most frequently studied. The benefit to this approach is that, in addition to acting as a protecting group, the tosyl moiety is also a good leaving group, enabling the synthesis of a variety of 6-deoxy-cellulose derivatives via nucleophilic displacement reactions. While tosylation is considered selective for *C*-6 on cellulose, it has been shown that substitution can also occur to some extent at the secondary hydroxyl groups of cellulose. Despite this lack of complete regioselectivity for the tosylation of cellulose, its frequent use in the synthesis of "regioselectively" derivatized cellulose compounds over the past two decades warrant its inclusion in this review.

There are reports of cellulose tosylation dating back several decades (Honeyman 1947; Malm, et al. 1948) in the literature, but use of the reaction in synthetic cellulose chemistry increased substantially after it was effectively demonstrated with a homogeneous solution of cellulose in DMAc/LiCl (Mccormick and Callais 1987). A subsequent investigation found that using triethylamine as an organic base in the reaction rather than pyridine resulted in a higher DS for the tosyl group and helped avoid side reactions that formed chlorodeoxycellulose (McCormick, et al. 1990). Recently, homogeneous tosylation of cellulose was also demonstrated in the ionic liquid 1-allyl-3-methylimidazolium chloride (Granström, et al. 2008).

Rahn et al. (1996) published an in depth study of the homogeneous tosylation of cellulose in DMAc/LiCl investigating how to control the total DS of the tosyl groups on cellulose, the solubilities of cellulose tosylates of varying DS, and the selectivity of the reaction for *C*-6. Cellulose tosylates with a DS from 0.4 to 2.3 were synthesized by increasing the equivalents of tosyl chloride added to the reaction. Those with a DS of about 0.9 or above were soluble in polar aprotic solvents such as DMSO, DMAc, and DMF. Increasing the DS to about 1.4 makes the derivatives also soluble in acetone, dioxane, and tetrahydrofuran. Further increasing the DS

above 1.8 expanded the solubility range of the derivatives to halogenated hydrocarbons like chloroform and dichloromethane. The selectivity of the tosylation reaction was evaluated by displacing the tosyl groups at C-6 with iodide. The analysis showed that tosylation occurred at *C*-6 almost exclusively on derivatives with a low DS (0.46), but that increasing the DS resulted in substitution occurring also at the secondary hydroxyl groups of cellulose. Tosylation of *C*-6 was nearly complete in derivatives with a DS of about 1.4. The graph in Figure 2.3, published by Rahn et al. (1996), shows the relationship between the partial DS at *C*-6 versus the total DS for the whole polymer. Therefore cellulose tosylation cannot be considered to be as selective for *C*-6 as the already mentioned tritylation and thexyldimethylsilylation reactions. Additionally, it was found that the iodination of the tosyl cellulose was not completely selective for *C*-6 either, as a small amount of iodide was substituted at the secondary hydroxyl groups as well. Therefore, while subsequent substitution reactions with tosyl cellulose probably occur primarily by an S_N2 type mechanism at *C*-6, it could be erroneous to assume that substitution takes place exclusively at that site.

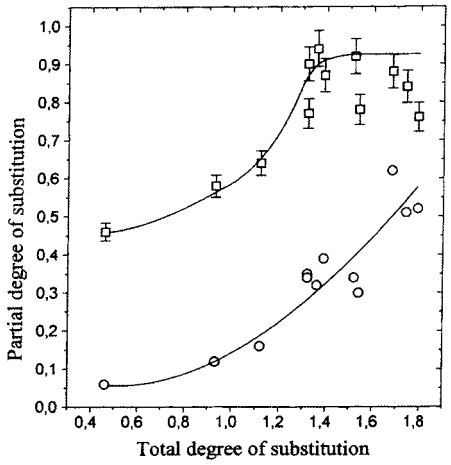


Figure 2.3 A graph published by Rahn et al. (1996) depicting the partial DS of tosylate groups at *C*-6 (square symbols) and at *C*-2/*C*-3 (round symbols) vs. the total DS of the tosylate group on cellulose. (Reprinted with permission from Rahn et al. (1996). Copyright 1996 Wiley-VCH Verlag GmbH & Co. KGaA.)

The thermal stability of tosyl celluloses with a DS from 0.4 to 2.3 was studied by Heinze et al. (1996b), and it was found that the compounds began to degrade at a temperature of 169 °C

to 196 °C. Tosyl cellulose was also investigated by dielectric spectroscopy to determine the influence of tosylate DS on the polymer chain mobility (Einfeldt, et al. 2002). The free hydroxyl groups of tosyl celluloses were reacted with a variety of aliphatic, aromatic and unsaturated acid anhydrides, as well as with isocyanates to form new compounds with a wide range of solubilities (Heinze, et al. 1996a).

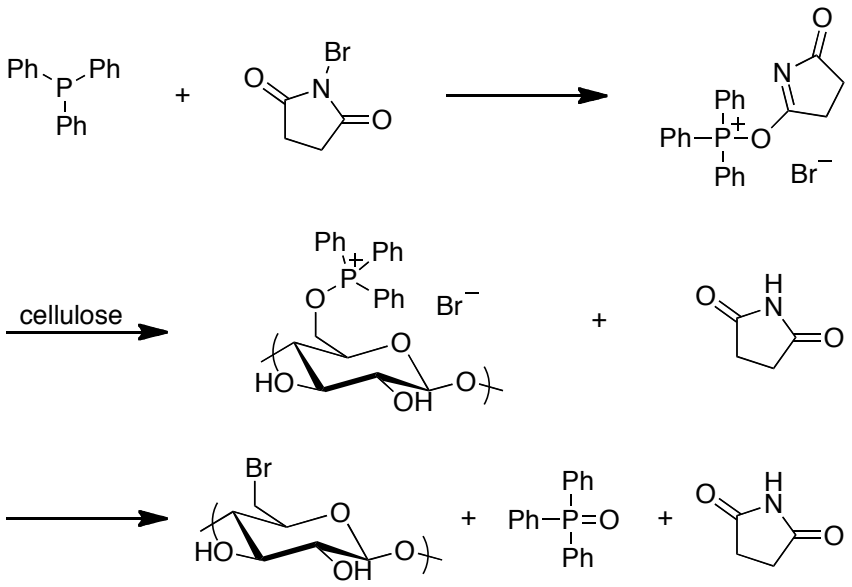
The most widely studied applications for tosyl cellulose have been for use in displacement reactions with nitrogen containing nucleophiles. A series of publications have examined the displacement of tosyl groups at *C*-6 of cellulose with aliphatic and aromatic diamines for the purpose of creating films capable of enzyme immobilization for biosensor applications (Tiller, et al. 1999; Berlin, et al. 2000; Tiller, et al. 2000; Tiller, et al. 2001; Berlin, et al. 2003; Becher, et al. 2004). Mais et al. (2000) synthesized cellulose-*graft*-poly(*N*-acetylethylenimine) from tosyl cellulose by reaction with 2-methyl-1,3-oxazoline. Bieser et al. (2011) recently published a study on the efficacy of using similar cellulose graft co-polymers for antimicrobial coatings. Chirality has been introduced to the C-6 position of cellulose by the substitution of tosyl groups with single enantiomers of 1-phenylethylamine (Heinze, et al. 2001). Water-soluble cationic cellulose derivatives were made by reacting tosyl cellulose with different trialkylamines (Koschella and Heinze 2001). In one interesting approach to synthesizing a truly regioselective cellulose derivative using tosyl cellulose, Liu and Baumann (2002) reacted cellulose that had been fully tosylated at *C*-6 with sodium azide. The resulting azido group at C-6 was then reduced with lithium aluminum hydride to a primary amine, and in the process reduced any tosyl esters at the *C*-2 and *C*-3 positions on cellulose back to free hydroxyl groups. The resulting 6-amino-6-deoxy-cellulose was then used to make both 6-*N*-sulfonated and 6-*N*-carboxymethylated cellulose. These derivatives were tested for their platelet adhesion properties for potential use as coatings for biomaterials (Baumann, et al. 2003). Liebert et al. (2006) also synthesized 6-azido-6-deoxy-cellulose from tosyl cellulose, but instead of reducing it to an amine, the azido groups were used as sites for Huisgen reactions (“click” chemistry). Using this chemistry, 1,4-disubstituted 1,2,3-triazoles can be used as linkers to the *C*-6 position for a wide variety of functional groups. Other nitrogen nucleophiles that have been reacted with tosyl cellulose include methylamine (Knaus, et al. 2003), butylamine, and pyridine (Liu and Baumann 2005). Pohl and Heinze (2008) reported reaction of 6-*O*-tosyl cellulose with DS = 0.58 (and with

high *C*-6 regioselectivity at that low DS(Ts)) with propargyl amine to give an alkyne precursor for click reactions that were employed to create dendritic structures.

Direct regioselective halogenation

Furuhata et al. (1992b) published a method to regioselectively halogenate cellulose at the *C*-6 position without going through an isolated intermediate like tosyl cellulose. This reaction required the dissolution of cellulose in DMAc/lithium *bromide*, followed by the addition of the reagents triphenylphosphine (Ph_3P) and *N*-bromosuccinimide (NBS). This reaction has a similar result to tosylation in that the bromide in the 6-deoxy-6-bromo-cellulose product can act as both a protecting group under certain conditions and as a good leaving group for substitution chemistry at the *C*-6 position. The advantage to this chemistry over tosylation is that the bromination is completely selective for *C*-6. That is, no measurable amount of bromination takes place at the secondary hydroxyl groups on cellulose. In addition, a maximum bromide DS of 0.98 resulting from this procedure has been reported, indicating that nearly every primary hydroxyl group on the cellulose backbone has been replaced by a bromide (Matsui, et al. 2005). Chlorination of cellulose dissolved in DMAc/LiCl with Ph_3P and *N*-chlorosuccinimide has also been reported, though it was found that chlorination also takes place at the secondary hydroxyl sites for a maximum DS of 1.86 (Furuhata, et al. 1992a).

The proposed mechanism (Furuhata, et al. 1992b) for the direct bromination of cellulose with Ph_3P and NBS is shown in Scheme 2.8. The reaction proceeds in three steps. First, Ph_3P and NBS react to form an alkoxyphosphonium salt. This salt then forms a new alkoxyphosphonium salt with cellulose, splitting off succinimide. The bromide anion can then attack the new alkoxyphosphonium salt, displacing triphenylphosphine oxide in the process. The regioselectivity of the reaction is likely derived from the steric bulk of the three benzene rings in the alkoxyphosphonium salt intermediates, which can only fit at a primary hydroxyl site, and the S_N2 type chemistry of the bromide ion displacing the triphenylphosphine. An S_N2 reaction is unfavored at the secondary hydroxyl groups on cellulose because of the furan structure of the glucose monomer restricting the backside approach of a nucleophile at *C*-2 and *C*-3. The inversion of configuration characteristic of an S_N2 reaction is also unlikely at *C*-2 and *C*-3 on cellulose since it would require movement of the entire cellulose chain.



Scheme 2.8 Proposed mechanism for bromination of cellulose at *C*-6 with Ph_3P and NBS (Furuhata, et al. 1992b)

The main disadvantage to regioselective bromination compared to tosylation is that the 6-deoxy-6-bromo-cellulose is not soluble in any typical aqueous or organic solvents, though it can be redissolved in DMAc/LiBr without first swelling or activating the cellulose. Nevertheless, several cellulose derivatives substituted with high regioselectivity at *C*-6 have been synthesized via nucleophilic displacement reaction with 6-deoxy-6-bromo-cellulose starting under heterogeneous conditions. Table 2.6 summarizes the cellulose derivatives that have been synthesized to date from 6-deoxy-6-bromo-cellulose. Reactions involving various thiols were shown to be effective in regioselectively attaching different pendant functional groups, such as carboxylic acids, dicarboxylic acids, and amines to the cellulose backbone. These reactions were run both heterogeneously in aqueous alkali (Aoki, et al. 1995) and homogeneously in DMAc/LiBr (Aoki, et al. 1996). Several of these derivatives were tested for their metal ion sorption capacities (Aoki, et al. 1999). 6-Deoxy-6-(2-cyanoethylamino)-cellulose and 6-deoxy-6-thiocyanato-6-cellulose were synthesized beginning with a heterogeneous suspension of 6-deoxy-6-bromo-cellulose in DMSO and DMF, respectively. The reactions became homogeneous as they progressed, and the products were used to study β-relaxations in cellulose by dielectric spectroscopy (Saad, et al. 1996; Saad and Furuhata 1997). Water soluble sulfonate derivatives were synthesized by reacting the brominated cellulose in aqueous solutions of sodium sulfite

(Furuhata and Ikeda 1999). A regioselectively substituted cationic cellulose derivative was produced by synthesis of 6-deoxy-6-azido cellulose from the brominated cellulose, followed by reduction of the azido group to a primary amine (Matsui, et al. 2005). The DS of the amine group on the cellulose was 0.96.

Table 2.6 Cellulose derivatives synthesized from 6-deoxy-6-bromo-cellulose

C-6 Substituent	DS[1]	Reference
Sulfur nucleophiles and derivatives		
Methanethiol	0.55	Aoki et al. 1995
Benzenethiol	0.41	Aoki et al. 1995
Mercaptoacetic acid	0.10	Aoki et al. 1995
L-Cysteine	0.56	Aoki et al. 1996
2-Mercaptoethanol	0.71	Aoki et al. 1996
3-Mercaptopropanoic acid	0.92	Aoki et al. 1996
2-Mercaptobutanedioic acid	0.51	Aoki et al. 1996
4-Aminobenzenethiol	0.84	Aoki et al. 1996
2-Aminobenzenethiol	0.76	Aoki et al. 1996
2-Mercaptobenzoic acid	0.64	Aoki et al. 1996
Thiocyanate	0.88	Saad and Furuhata 1997
2-Aminoethanethiol	0.79	Aoki et al. 1999
Isothiourea	0.80	Aoki et al. 1999
Mercaptan	0.79	Aoki et al. 1999
Sodium sulfonate	0.75	Furuhata and Ikeda 1999
Nitrogen nucleophiles and derivatives		
2-Cyanoethylamine	0.86	Saad et al. 1996
Azide	0.96	Matsui et al. 2005
Primary amine	0.96	Matsui et al. 2005

[1] The highest DS for the derivative reported in the literature is the DS recorded in this table

Conclusions

The different methods for regioselective synthesis of cellulose derivatives have been presented here. The most commonly used methods involve the use of protecting groups, specifically the trityl group used to protect the *C*-6 hydroxyl group and the thexyldimethylsilyl group used to protect either the *C*-6 hydroxyl group or both the *C*-6 and *C*-2 hydroxyl groups at the same time. These methods, along with the *de novo* synthesis of derivatives starting from glucose orthopivalate esters, have enabled the production of highly regioselectively substituted alkyl ether derivatives of cellulose for structure/property relationship studies. It has become clear from these studies that the position of substitution for cellulose derivatives affects the solubility and thermal properties of the products. As a result, regioselective cellulose chemistry can be used as a tool to access tighter control over the physical properties of cellulose derivatives. This control may prove useful when making cellulose derivatives for advanced applications, such as in drug delivery (see next section).

Regioselective synthesis of cellulose derivatives can be a laborious task requiring numerous reaction steps. The use of protecting groups inevitably requires a deprotection step in the synthesis scheme to obtain the desired derivative. *De novo* syntheses generally require even more reaction steps than protecting group strategies, and they can also only achieve relatively low molecular weight polymers. The use of functional groups that can activate certain hydroxyl groups on cellulose to a wider spectrum of reactions offers the chance of a less laborious procedure for regioselective derivatization of cellulose. To date, this strategy has only been successful for *C*-6 using tosylation and direct bromination reactions. Of these two reactions, bromination is far more selective for *C*-6 than tosylation. However, it is also the less studied of the two, and further investigation is needed to develop that strategy as another alternative for regioselective synthesis of cellulose derivatives.

Cellulose Derivatives in Oral Drug Delivery

Several avenues exist for introducing pharmaceutical drugs into the human body, each with its own set of unique advantages and disadvantages. However, oral administration is the route most preferred by patients for its convenience, familiarity, and lack of discomfort. Taking a pill by mouth does not require the presence of a medical professional as injections typically do,

and the dreaded pain of the needle can be avoided. Other routes of drug administration, such as by transdermal absorption or inhalation, can also be self-administered and generally avoid discomfort, but they are limited to relatively few applications by available technology and the chemical nature of the drugs themselves. Oral formulations, on the other hand, are available for a wide spectrum of drugs. In addition, drugs for oral administration are usually less expensive to manufacture than other types of formulations. Table 2.7 gives a comparison of a few common routes of drug administration. A more detailed review of drug administration is given by Jain (2008).

Table 2.7 Comparison of common methods for drug administration

Route of Administration	Advantages	Disadvantages
Oral	High patient acceptance Self-administered Low discomfort Used for a broad spectrum of drugs	Potential for degradation of drugs in the GI tract Low bioavalability for drugs with poor aqueous solubility Low bioavalability for drugs with poor membrane permeability 1st pass metabolism
Injections	High bioavailability High control over dosage Rapid onset of action	Generally requires presence of nurse or doctor Discomfort to the patient
Transdermal	Self-administered Sustained delivery of drug	Limited to lipophilic drugs that can penetrate the skin Potential for skin irritation
Inhalation	Rapid drug absorption and action High control over dosage Self-administered	Requires rigid control of aerosol droplet size

Despite its advantages, however, oral administration can lead to problems with the bioavailability of a drug. Bioavailability is a term describing the fraction of the administered drug that makes it into the bloodstream intact and can be presented to the site of action within the body. Thus, if a pill containing 100 mg of a drug is swallowed and only 20 mg of the drug is absorbed intact into the bloodstream, the oral bioavailability for that drug is 20%. An orally administered drug is presented with several obstacles as it traverses the gastrointestinal (GI) tract that can prevent it from being absorbed into the bloodstream, thus reducing its bioavailability. After being swallowed, the drug travels through the esophagus to the stomach, where it must be stable in the low pH gastric fluids and among protease enzymes (see Figure 2.4 and Table 2.8). From the stomach, it passes to the more neutral pH environment of the small intestine. Here, the architecture of the intestinal epithelium creates a large surface area through which drug molecules can be absorbed into the bloodstream. However, in order for absorption to occur, the drug must have the ostensibly contradictory properties of high enough hydrophilicity to dissolve in the intestinal fluids and high enough lipophilicity to pass through the epithelial membrane. In addition, the drug molecules must not be substrates for transport enzymes designed to reject foreign molecules from enterocytes back into the intestinal lumen. Drug molecules that are either not absorbed through the intestinal epithelia or rejected back into the GI tract will eventually be cleared out of the body through the anus, never reaching circulation in the bloodstream. Molecules that are absorbed and remain in the bloodstream are then subjected to first pass metabolism in the liver, a process that takes foreign substances in the blood and converts them to a chemical form that is more easily cleared from the body through the kidneys. If a drug molecule can survive this process intact, then it can finally enter wider circulation within the body. An in depth review of the mechanisms by which drug molecules can pass through GI tract membranes and into the bloodstream, as well as the metabolic processes drug molecules must survive, is given by Calcagno and Siahaan (2005).

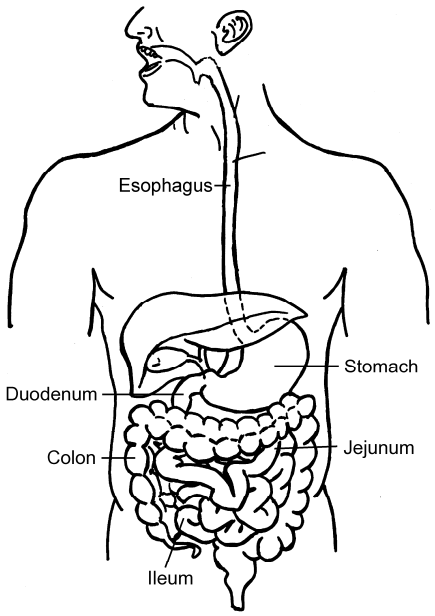


Figure 2.4 Illustration of the human GI tract. (Reprinted with permission from Klein, S., Polysaccharides in Oral Drug Delivery - Recent Applications and Future Perspectives. In *Polysaccharide Materials: Performance by Design*, American Chemical Society: 2009; Vol. 1017, pp 13-30. Copyright 2009 American Chemical Society.)

Table 2.8 Characteristics of GI tract segments (Kendall and Basit 2006)

Segment in GI tract	Approx. surface area	Approx. length	Residence time	Approx. pH
Stomach	0.1 m^2	0.20 m	variable	1 – 2.5
Duodenum	1.9 m^2	0.25 m	↓	5.5 – 6.0
Jejunum	184 m^2	2.8 m	3 – 5 h total	6.0 – 7.0
Ileum	276 m^2	4.2 m	↑	7.0 – 7.5
Colon	0.25 m^2	1.5 m	20 – 30 h	7.0 - 7.5

Overcoming problems with variability in drug absorption from the GI tract into the bloodstream and increasing bioavailability of drugs is a major focus area in drug delivery research. Cellulose derivatives have played an important role in the development of technologies to help with these issues. Among the important properties of the cellulose derivatives currently used for drug delivery applications are their negligible toxicity and stability in the human gut, their good water permeability, and the high stiffness of the polymer chains. The remainder of this

review will discuss three applications of cellulose ethers and esters that are used in oral drug formulations, and how their physical properties help to improve drug performance. This section is not a comprehensive literature review, but it is intended to demonstrate the utility of cellulose derivatives in oral drug delivery.

Sustained release of orally administered drugs

Sustained release formulations are commonly used in oral drug delivery to prolong the release of water-soluble drugs within the GI tract. The benefits of this type of formulation include reducing the frequency with which the drug has to be administered, thus increasing the convenience for the patient, and minimizing fluctuations of the drug concentration in plasma over time (Tiwari and Rajabi-Siahboomi 2008). One method for obtaining sustained release is by surrounding the drug with a water permeable membrane. The aqueous fluids in the GI tract must then diffuse across the membrane in order to dissolve the drug, limiting its rate of dissolution. Next, the solvated drug must diffuse back across the membrane to be released into the body. Thus, polymers to be used to form such membranes must necessarily be water permeable. Several cellulose derivatives that can form water permeable films and matrices are widely used for sustained drug release.

Hydroxypropylmethylcellulose (HPMC), also referred to as hypromellose, is a cellulose ether derivative that is commonly used in drug formulations for its ability to form hydrophilic gels. A review of the literature regarding the use of HPMC in sustained release forms, including drug release mechanisms and release rates, has been published by Li et al. (2005). Oral formulations with HPMC are usually manufactured by pressing a tablet of a mixture of a powder of the polymer, the drug, and other excipients. When the HPMC comes in contact with water, it forms a gel through which the drug molecules must pass to be released. Over time, the outer layer of the HPMC gel dissolves in water, allowing a secondary mechanism for drug release by membrane erosion (Colombo, et al. 1996). The release rate of the drug is affected by such factors as the drug solubility, the concentration of the polymer, the viscosity of the polymer, and the inclusion of other excipients in the formulation (Tahara, et al. 1995). At low polymer to drug ratios, it has been reported that gels formed from different HPMC polymers with varying DS levels for the methyl and hydroxypropyl substituents resulted in different drug release profiles (McCrystal, et al. 1999). Measurements on the diffusion of water across HPMC gel membranes

using NMR spectroscopy suggested that this could be due to differences in water diffusion rates (Rajabi-Siahboomi, et al. 1996). Interestingly, HPMC matrices have been observed to have some chiral-selective properties in sustained release formulations. Small differences in the release rate of the enantiomers of propranolol hydrochloride (Duddu, et al. 1993) and ketoprofen (Solinís, et al. 2002) have been reported.

Water insoluble cellulose derivatives such as ethyl cellulose, cellulose acetate, or mixed cellulose esters (e.g. cellulose acetate propionate and cellulose acetate butyrate) are also commonly used in sustained release applications (Edgar, et al. 2001; Edgar 2007; Klein 2009). Since these polymers do not erode away over time, the release of the drug is dependent on the diffusion of aqueous fluids across the membranes. The simplest method for forming the matrices is again by direct compression of physical mixtures of drug and polymer, though polymer coated particles containing a drug can also be made by co-precipitation of the polymer and drug from a common solution into a common anti-solvent. Edgar et al. (2001) discussed several examples of sustained release formulations manufactured by compression of drugs and cellulose esters into tablets. Cellulose acetate was used as a sustained release matrix for acetaminophen, theophylline, dyphilline, and proxyphylline at high drug to polymer ratios. Increasing the amount of cellulose acetate relative to the drugs further prolonged the release of the drugs as expected. The molecular weight of the cellulose acetate was not found to have a significant effect on the release rate. A relatively new cellulosic polymer, carboxymethylcellulose acetate butyrate (CMCAB), was combined in physical mixtures with aspirin, ibuprofen, or fexofenadine HCl. The mixtures were shown to provide a near constant rate of drug release *in vitro* over several hours (Posey-Dowty, et al. 2007). Several studies on the formation of microparticles by co-precipitation of a drug and mixed cellulose esters were reviewed by Edgar (2007). The interest in reducing the particle size in these studies was driven by evidence that smaller particles result in lower variability and better performance once administered. They also allow for more versatility in manufacturing drug formulations.

Another strategy for attaining sustained drug release is by using osmotic pump delivery systems (see Figure 2.5 for an illustration). In this type of formulation, a combination of a drug and salt are encapsulated by a water permeable membrane. Once ingested, water diffuses across the membrane and dissolves the salt and drug. Since the salt cannot readily penetrate the membrane, an osmotic pressure gradient is built up across the membrane. This gradient then

forces the dissolved drug out through a small laser drilled hole in the membrane at a constant rate. Cellulose esters, especially cellulose diacetate, are commonly used as the membrane material in osmotic pump formulations because of their film strength, water permeability, and lack of toxicity. The permeability of cellulose ester membranes, and thus the rate at which the drug is released, can also be controlled by adjusting the DS of ester groups on the cellulose backbone (Edgar 2007).

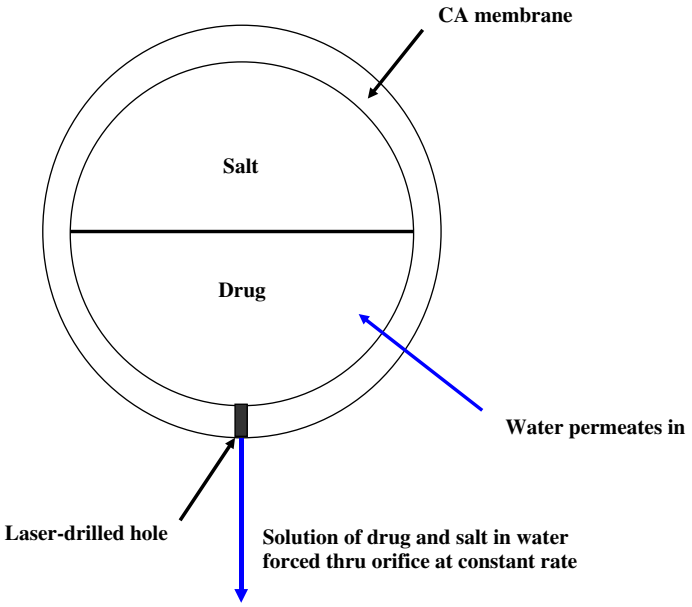


Figure 2.5 Depiction of an osmotic pump delivery system. (Reprinted with kind permission from Springer Science+Business Media: *Cellulose*, Cellulose esters in drug delivery, 14, 2007, pp. 49-64, KJ Edgar, Figure 5.)

Enteric coatings

Drugs that are sensitive to acidic conditions can be coated with pH responsive polymers to protect them from degradation in the stomach. Conversely, these enteric coatings can also protect the stomach lining from irritation caused by the drugs. Polymers that respond to changes in pH are desirable so that after protecting the drug in the stomach, they release the drug in the higher pH environment in the small intestine. This behavior is typically provided by polymers with pendant carboxylic acid functional groups. In the stomach, where the pH is below the pK_a of the carboxylic acids, the polymer is protonated and insoluble in the gastric fluids. In the

intestines, where the pH is above the pK_a of the carboxylic acids, the carboxylic acids ionize and cause the polymer to swell or dissolve, thus releasing the drug.

Cellulose acetate phthalate (C-A-P) was among the first polymers investigated for its pH-controlled release properties (Edgar 2007). C-A-P with a phthalate DS of 0.8 is soluble at a pH of about 5.8 and has good compatibility with organic solvents and plasticizers. Other cellulosic polymers that have been studied for use as enteric coatings include cellulose acetate trimellitate (C-A-T), cellulose acetate succinate (CASu), hydroxypropyl methylcellulose phthalate (HPMCP), hydroxypropyl methylcellulose acetate succinate (HPMCAS), and CMCAB (Yang, et al. 2004; Edgar 2007; Posey-Dowty, et al. 2007). The rate of drug release from these polymers is dictated by the pK_a of their respective carboxylic acid groups, the pH of the aqueous media, and the hydrophilicity of the polymers as demonstrated by the graph in Figure 2.6. The low pK_a of C-A-T resulted in a quicker release of aspirin than was observed for C-A-P and HPMCP at a pH of 5.2. However, at a pH of 6.2, all three polymers had a similar release profile. The increased hydrophilicity of HPMCP imparted by the hydroxypropyl groups allowed the release of aspirin at a pH of 5.2, where the same conditions resulted in almost zero drug release from C-A-P.

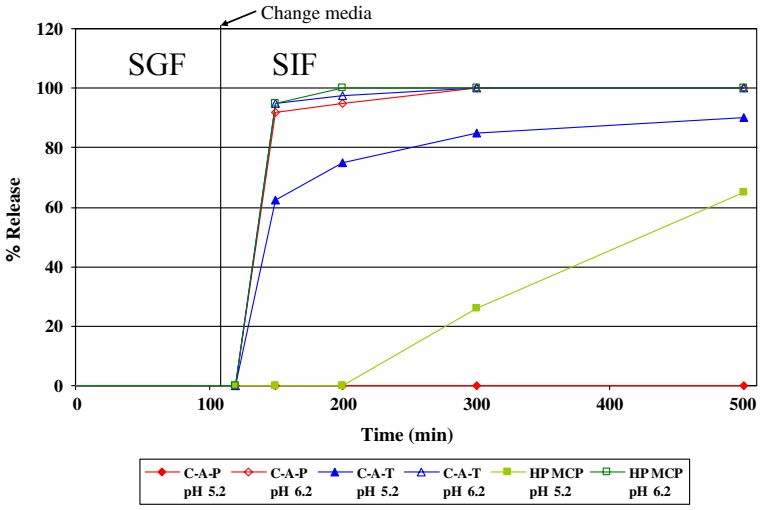


Figure 2.6 The effect of pH on aspirin release for C-A-P, C-A-T, and HPMCP (SGF = simulated gastric fluid; SIF = simulated intestinal fluid). (Reprinted with kind permission from Springer Science+Business Media: *Cellulose*, Cellulose esters in drug delivery, 14, 2007, pp. 49-64, KJ Edgar, Figure 1.)

Solid dispersions

For many drugs, poor aqueous solubility is the major factor contributing to their low oral bioavailability, and it is widely recognized that modern drug discovery techniques are resulting in more molecules with poor solubility being investigated as drug candidates (Dahan and Amidon 2009). Consequently, getting these drugs to dissolve in the GI tract is a significant challenge to improving drug efficacy. One technique that has been used to address this issue is dispersing the drug in an amorphous polymer matrix. This can be accomplished by dissolving both the drug and polymer in a common organic solvent, followed by either removal of the solvent by evaporation (for example, by film casting or spray-drying) or co-precipitation of the drug and polymer in a common anti-solvent. If the drug and polymer have adequate miscibility, the drug will be dispersed on a molecular level within the polymer, and any drug crystallinity will be disrupted. Once again, water permeability is a desirable trait of the polymer so that the solvated drug can diffuse out of the matrix after it has been ingested. In addition, high polymer chain stiffness is beneficial for physically holding the drug molecules apart and stabilizing the dispersion during storage. Since cellulose forms a very stiff, hydrophilic polymeric backbone, several cellulose derivatives have been investigated for making solid dispersions of drugs. The ability to attach different functional groups onto cellulose is also advantageous since the properties of cellulose derivatives can be tailored to increase the specific interactions between a polymer and a drug, improving their miscibility.

Films of C-A-P, HPMCP, and HPMC containing the antibiotic erythromycin were studied by Sarisuta et al. (1999). The authors found that the drug was molecularly dispersed in all three polymers even after subjecting the films to temperature cycles between 8 and 40 °C for several days. A similar study with the drug felodipine cast in films of HPMCAS and HPMC found that the polymers decreased the nucleation rate of the drug (Konno and Taylor 2006). In a related paper, HPMCAS and HPMC were investigated for their ability to inhibit recrystallization of felodipine from a supersaturated solution after the drug had been released from the amorphous polymer matrix (Konno, et al. 2008). While both polymers were found to inhibit recrystallization, HPMCAS was determined to be the better choice for stabilizing supersaturated solutions of the drug. The mechanism by which polymers inhibit drug crystallization from supersaturated solutions is not understood and requires further investigation. In another study, HPMCP was co-precipitated with an experimental drug from GlaxoSmithKline, and the amount of the drug

incorporated into the polymer matrix was studied as a function of variables such as antisolvent to solvent ratio, antisolvent temperature, stirrer speed, antisolvent pH, etc. (Sertsou, et al. 2002). The significant factors in their experiment were determined to be the amount of the drug used, the stirrer speed, and the antisolvent pH. Particles of HPMCP and HPMCAS containing the drug nitrendipine were produced by Yang et al. (2004). Again, the drug formed an amorphous dispersion within both polymers, and the dispersions were physically stable after being stored for 3 months at 40 °C and 75% relative humidity. *In vivo* studies in dogs suggested that the formulations improved the bioavailability of nitrendipine. Friesen et al. (2008) produced amorphous dispersions of nine different drugs and drug candidates in HPMCAS by spray-drying from solution. *In vitro* tests showed in each case that the dispersions resulted in more drug going into solution than adding the drug alone to the aqueous solvent. The solid dispersions were also shown to result in higher bioavailability for four of the drugs in *in vivo* experiments with dogs.

Concluding remarks

The utility of cellulose ethers and esters in the three oral drug delivery applications discussed here has been well-established in the literature. These cellulose derivatives do not have to serve single, discreet functions when included in oral formulations, but can serve several purposes at once. For example, CMCAB and HPMCAS can be used as enteric matrices, provide sustained release of a drug, and improve the drug's solubility all in one formulation (Posey-Dowty, et al. 2007; Friesen, et al. 2008). The role of these polymers in oral drug delivery is likely to become even more important in the future, especially as the fraction of drug candidates that have poor aqueous solubility increases.

Despite all the history of cellulose and oral drug delivery, there have been no studies published investigating regioselectively substituted cellulose derivatives in drug formulations. Since regioselectivity in cellulose reactions is known to affect the solubility properties of the resulting cellulose derivatives, developing regioselectively substituted cellulose derivatives for drug delivery may result in better control over the properties of oral drug formulations. Fine-tuning the miscibility of specific drugs with an amorphous matrix polymer could potentially allow researchers to optimize the rate and location at which those drugs are released in the GI tract for a given therapy. With a number of pathways now available for synthesizing regioselective cellulose derivatives, this is a topic that is ripe for research.

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CHAPTER 3: REGIOSELECTIVE SYNTHESIS OF ORGANIC SOLUBLE 6-BROMO-6-DEOXY-CELLULOSE ESTERS

Adapted with kind permission from Springer Science+Business Media: *Cellulose*, Synthesis of regioselectively brominated cellulose esters and 6-cyano-6-deoxycellulose esters, 18, 2011, pp. 1305-1314, SC Fox and KJ Edgar.)

Abstract

A highly regioselective one-pot reaction scheme has been devised to synthesize 6-bromo-6-deoxy-2,3-di-*O*-acyl-cellulose from microcrystalline cellulose. These halogenated cellulose esters are readily soluble in many common organic solvents, including acetone, dimethyl sulfoxide, dimethylformamide, tetrahydrofuran, and chloroform. Analysis of the products by ^{13}C NMR indicates that little or no bromide substitution occurs at the secondary hydroxyl groups, and comparison of their molecular weights to that of the starting cellulose shows little degradation of the polysaccharide backbone. Thus, this method represents an improvement over other methods used to activate *C*-6 in cellulose, such as the less regioselective tosylation reaction. The chemical structure of the 6-bromo-6-deoxy-cellulose esters was further confirmed by FTIR, ^{1}H NMR, and elemental analysis. Thermal properties were also measured by DSC and TGA.

Introduction

Halogenated cellulose derivatives are of interest to cellulose chemists as potential intermediates for the preparation of new functional materials. Halogen atoms substituted for the hydroxyl groups at carbons 2, 3, and 6 (*C*-2, -3, and -6; see Figure 2.1) on each AGU in cellulose can allow for the participation of those carbon sites in reactions with nucleophiles since the halogen can act as a leaving group. Thus, a wide variety of functional groups could then theoretically be attached to the cellulose backbone via substitution chemistry.

A review by Krylova (1987) discusses several methods developed to chlorinate cellulose, some of which can result in high chlorine DS. Treatment of a suspension of cellulose in

chloroform with sulfuryl chloride and pyridine can result in chlorodeoxycellulose with a DS_{Cl} up to 1.7. The review cites Russian literature from the 1980's that reports the reaction occurs first at the primary hydroxyl groups at *C*-6, followed by chlorine substitution at *C*-3 with inversion of configuration. Interestingly, the claim is made that no substitution occurs at *C*-2. A similar study was reported by Furubeppu et al. (1991) in which cellulose dissolved in dimethylacetamide/lithium chloride (DMAc/LiCl) was reacted homogeneously with sulfuryl chloride. A DS_{Cl} of 1.8 was obtained, and gas chromatographic analysis of the acid hydrolyzate of the product again indicated that substitution occurs only at the *C*-6 and *C*-3 positions. The Krylova review goes on to discuss the reaction of cellulose dissolved in a dimethylformamide (DMF)/chloral solvent system with thionyl chloride to produce chlorodeoxycellulose with a DS_{Cl} of 2.8. It also discusses regioselectively synthesized 6-chloro-6-deoxy-cellulose derivatives produced by the introduction of acetyl or carbanilate protecting groups at the secondary hydroxyl sites prior to halogenation.

An alternative to the halogenation of cellulose for activating carbon atoms to substitution chemistry is tosylation. Tosylation reactions are similar to halogenation reactions in the sense that tosyl groups are also good leaving groups for subsequent substitution chemistry. It is also known that tosylation on cellulose occurs preferentially at the *C*-6 position, allowing a mechanism by which new functional groups can be selectively substituted for the primary hydroxyl groups along the cellulose backbone. However, it has been shown that significant tosyl substitution also occurs at the secondary hydroxyl groups (see Figure 2.3). The removal of these tosyl groups to regenerate free hydroxyl groups can be difficult, usually requiring a strong reducing agent such as lithium aluminum hydride. The tosylation of cellulose is discussed in more detail in both Chapter 2 and Chapter 4 of this dissertation, and in the paper by Rahn et al. (1996).

In 1992, Furuhata et al. (1992a; 1992b) published two papers on new methods to halogenate cellulose. These papers described the homogeneous chlorination and bromination of cellulose using triphenylphosphine (Ph_3P) and either *N*-chlorosuccinimide (NCS) or *N*-bromosuccinimide (NBS) as reagents. The maximum DS_{Cl} achieved for the chlorination reaction was 1.86, again with substitution occurring only at the *C*-6 and *C*-3 sites. The DS_{Br} achieved by the bromination reaction was 0.9, with chromatographic analysis of the acid hydrolyzate of the product indicating that the reaction was completely selective for *C*-6. This was a significant

finding because up to that point, the only way to achieve a high halogen DS and maintain high regioselectivity at *C*-6 on cellulose was to protect the secondary hydroxyl groups prior to halogenation, a process that requires a fairly arduous, multi-step synthetic procedure. More recently, a DS_{Br} of 0.98 has been reported using the same Ph_3P/NBS method while still maintaining very high regioselectivity for *C*-6 (Matsui, et al. 2005).

However, the solubility of 6-bromo-6-deoxy-cellulose is not significantly improved from that of unmodified cellulose. This lack of solubility can hinder its reactivity in subsequent nucleophilic reactions and limit the conversion of the bromide substituent to a new substituent. Reactions in which high conversion is desired must either lead to reactive dissolution (Saad, et al. 1996; Aoki, et al. 1999; Matsui, et al. 2005) or begin with re-dissolving the starting material in DMAc/LiBr (Furuhata and Ikeda 1999; Aoki, et al. 1996). Our interest in using regioselectively halogenated cellulose for subsequent transformations into new cellulose derivatives led us to examine this problem. A simple solution, described in this chapter, was found by acylating the unsubstituted secondary hydroxyl groups. This was accomplished *in situ* by adding the acylating reagent directly to the halogenation reaction solution, thus providing a one-pot reaction to produce 6-bromo-6-deoxy-2,3-di-*O*-acyl-cellulose. These new ester derivatives exhibited good solubility in a range of organic solvents and were found to be useful intermediates for the synthesis of other cellulose derivatives selectively substituted at *C*-6 as described in subsequent chapters.

Experimental

Materials

Microcrystalline cellulose (MCC, Avicel® PH-101, Fluka) was dried under reduced pressure at 50 °C overnight prior to use. Lithium bromide (LiBr, Fisher) and sodium cyanide (NaCN, Sigma) were dried under reduced pressure at 125 °C. N-Bromosuccinimide (NBS, Sigma) was recrystallized from boiling water and dried for two days under reduced pressure over anhydrous calcium chloride. Triphenylphosphine (Ph_3P, Strem), all carboxylic acid anhydrides (Acros), and phenyl isocyanate (Acros) were used as received. Dimethylacetamide (DMAc,

Fisher) and dimethyl sulfoxide (DMSO, Acros) were kept over 4 Å molecular sieves and stored under dry nitrogen until use. Acetone and methanol (Fisher) were used as received.

Measurements

^{13}C NMR spectra were obtained on Varian INOVA or UNITY 400 MHz spectrometers with a minimum of 5,000 scans in either $CDCl_3$ or d_6-DMSO. Chemical shifts are reported relative to the solvent peaks. A Thermo Electron Nicolet 8700 FTIR was used to perform infrared spectroscopic analysis of the samples as pressed KBr pellets. Molecular weight determination was achieved by gel permeation chromatography in N-methylpyrrolidone containing 0.05% lithium bromide using a Waters 1515 isocratic HPLC pump, Viscotek 270 dual detector, and Waters 2414 refractive index detector. Universal calibration curves were prepared using polystyrene standards. All elemental analyses were performed by Atlantic Microlab, Inc. Carbon, hydrogen, and nitrogen contents were determined using either Perkin Elmer 2400 II or Carlo Erba 1108 elemental analyzers. Bromine contents were determined by flask combustion followed by ion chromatography. Differential scanning calorimetry (DSC) was performed on a TA Instruments Q2000 DSC. Each run consisted of a 10 °C/min heating ramp from 30 °C to 200 °C, followed by a 10 °C/min cooling ramp back to 30 °C, and then a second heating ramp at 10 °C/min to 200 °C. Thermal transitions were recorded from the second heating cycle. Thermal gravimetric analysis (TGA) was performed using a TA Instruments Q500 TGA using a 10 °C/min temperature ramp to 600 °C under a nitrogen purge gas.

Dissolution of MCC in DMAc/LiBr

Dried MCC (5 g, 30.8 mmol anhydroglucose units (AGU)) was weighed into a one liter, three-necked round-bottom flask. The flask was fitted with a nitrogen inlet, thermometer, and an overhead stirrer. Next, 225 mL DMAc was added to the flask, and the contents were stirred. The flask was flushed with dry nitrogen and heated in an oil bath to 160 °C. As the flask was heated, 25 mL of the DMAc was distilled under a stream of nitrogen. The slurry was kept at 160 °C for 1 h, after which it was allowed to cool to 90 °C. Next, LiBr (45 g, 0.518 mol) was added to the flask and dissolved in the DMAc. The contents of the flask were allowed to cool to room

temperature while being stirred continuously. The MCC dissolved within 2 h to form a transparent solution. All cellulose solutions were kept under dry nitrogen until use within 24 h.

Synthesis of 6-bromo-6-deoxy-2,3-di-*O*-acyl-cellulose

Ph_3P (32.35 g, 4 eq per AGU) was dissolved in 100 mL dry DMAc. Then NBS (21.95 g, 3 eq per AGU) was dissolved in an additional 100 mL dry DMAc. The Ph_3P solution was added to the MCC solution dropwise via a liquid addition funnel. The NBS solution was added likewise after the addition of the Ph_3P solution. The reaction solution was then heated to 70 °C, and the reaction was allowed to continue for 1 h. Esterification of the remaining hydroxyl groups was accomplished by the dropwise addition of 5 eq of a carboxylic acid anhydride (acetic, propionoic, or butyric anhydride) followed by continued reaction overnight at 70 °C. The reaction was halted by allowing it to cool to room temperature and then adding it slowly to 4 L of a 50:50 mixture of methanol and deionized water, precipitating the product. The precipitate was recovered by filtration and then re-dissolved in acetone. The acetone solution was concentrated under reduced pressure, and the product was reprecipitated in ethanol. The isolated product was dissolved in acetone and reprecipitated in ethanol one more time, and then it was dried under reduced pressure at 50 °C.

Carbanilation of microcrystalline cellulose

Samples of the MCC used in the above experiments were derivatized according to a previously published procedure (Evans, et al. 1989) to prepare them for GPC analysis. Briefly, 100 mg dried MCC was suspended in 20 mL anhydrous pyridine. Then, 2 mL phenyl isocyanate was added to the flask. The mixture was heated under nitrogen in an oil bath at 80 °C for 24 h, during which time the MCC completely dissolved. To halt the reaction, 2 mL methanol was added. The solution was allowed to cool, and then it was added to 200 mL 30/70 methanol/water. The resulting precipitate was filtered out and thoroughly washed with additional 30/70 methanol/water. The product was then dried in a vacuum oven at 50 °C.

Results and Discussion

Bromination of microcrystalline cellulose

Microcrystalline cellulose was dissolved in DMAc/LiBr and regioselectively brominated at *C*-6 using the Ph_3P and NBS reagents in a procedure similar to that first reported by Furuhata et al. (1992b). In the proposed mechanism for this reaction (see Scheme 2.8), the Ph_3P preferentially forms an alkoxyphosphonium salt intermediate at the primary hydroxyl group attached to *C*-6 due to the steric bulk of the three phenyl rings. A bromide anion, supplied by the NBS, then attacks *C*-6 via an S_N2 mechanism with triphenylphosphine oxide as the leaving group. This likely results in further selectivity for the primary hydroxyl site on cellulose due to the difficulty of backside attack and inversion of configuration at the secondary hydroxyl sites. The result is a reaction forming 6-bromo-6-deoxycellulose as the product with near complete regioselectivity for the bromide substitution.

The problem of poor solubility of 6-bromo-6-deoxy-cellulose was overcome in this study by the *in situ* esterification of the remaining free hydroxyl groups. This was accomplished by the addition of acetic, propionic, or butyric acid anhydride *in situ*, without isolation of the intermediate 6-bromo-6-deoxycellulose. Thus, 6-bromo-6-deoxy-2,3-di-*O*-acyl-celluloses were synthesized via a one-pot reaction scheme from microcrystalline cellulose. The FTIR spectra for 6-bromo-6-deoxy-2,3-di-*O*-acetyl-cellulose, 6-bromo-6-deoxy-2,3-di-*O*-propionyl-cellulose, and 6-bromo-6-deoxy-2,3-di-*O*-butyryl-cellulose are shown in Figures 3.1, 3.2, and 3.3, respectively. Variation in the length of the acyl chain did not significantly impact the spectra other than in the intensity of the aliphatic C-H stretching absorptions between 2750 cm^{-1} and 3100 cm^{-1}. The small or non-existent broad hydroxyl stretching absorption near 3500 cm^{-1} in the FTIR spectra of the products indicated that nearly all of the free hydroxyl groups had been substituted, and the introduction of ester groups is evidenced by the strong carbonyl absorption at 1760 cm^{-1}. The relatively weak absorption near 550 cm^{-1} in this spectrum also supports formation of the 6-bromo derivative, though elemental and NMR analysis of the product (discussed in detail below) are more conclusive evidence of the extent of bromination. The products were readily soluble (Table 3.1) in various organic solvents, including acetone, acetylacetone (Acac), DMSO, DMAc, dimethylformamide (DMF), chloroform ($CHCl_3$), and tetrahydrofuran (THF). In comparison, tosylated cellulose must have a DS of near 1.8, where a considerable number of secondary hydroxyl groups have also been substituted, to achieve similar solubility across this range of

solvents (Rahn, et al. 1996). It should be noted, however, that the effect of the molecular weight of the brominated cellulose esters on their solubility has not yet been investigated. The brominated cellulose esters were not soluble in diethyl ether, toluene, methanol, or ethanol. This improved solubility simplifies both the analysis of the halogenated cellulose and subsequent reactions with various nucleophilic reagents.

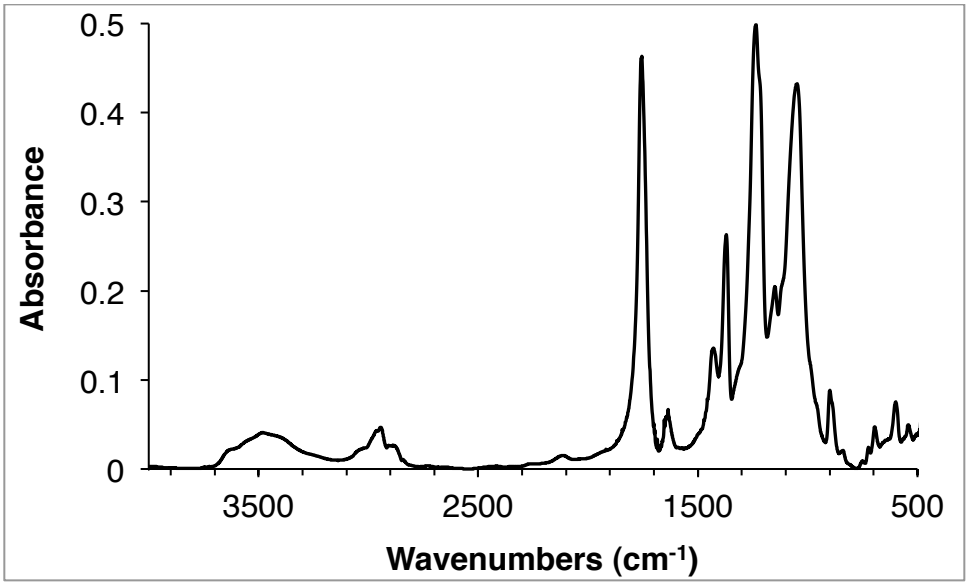


Figure 3.1 FTIR spectrum of 6-bromo-6-deoxy-2,3-di-*O*-acetyl-cellulose

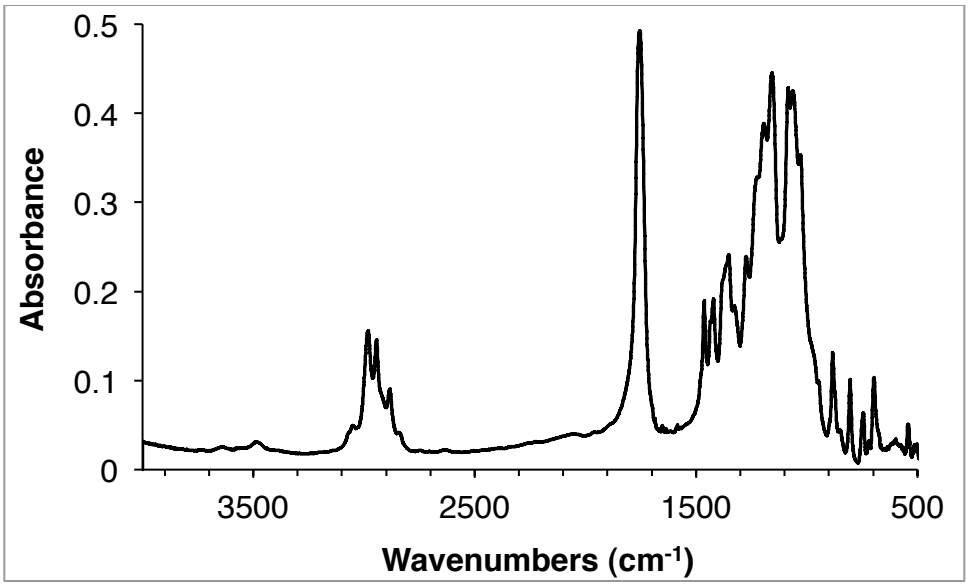


Figure 3.2 FTIR spectrum of 6-bromo-6-deoxy-2,3-di-*O*-propionyl-cellulose

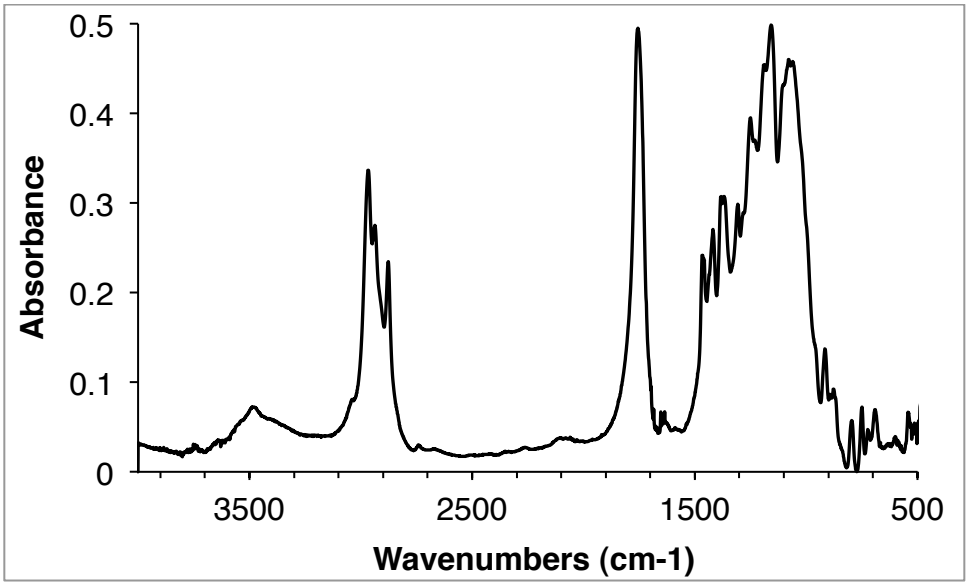


Figure 3.3 FTIR spectrum of 6-bromo-6-deoxy-2,3-di-*O*-butyryl-cellulose

Table 3.1 Solubility of brominated cellulose, brominated cellulose esters, and cellulose tosylates in various solvents[1,2,3]

	Acetone	Acac	DMSO	DMF	THF	$CHCl_3$	Toluene	Ethanol
6-Br-cellulose	-	-	-	-	-	-	-	-
6-Br-cellulose esters[4]	+	+	+	+	+	+	-	-
Cellulose tosylate (DS = 0.93)	⊕	-	+	+	⊕	-	(NIA)	(NIA)
Cellulose tosylate (DS = 1.79)	+	+	+	+	+	+	(NIA)	(NIA)

1) Acac = acetylacetone, DMSO = dimethyl sulfoxide, DMF = dimethylformamide, THF = tetrahydrofuran, $CHCl_3$ = chloroform

2) + Soluble; - Insoluble; ⊕ Swells; (NIA) no information available

3) Cellulose tosylate solubility data from Rahn et al. (1996)

4) 6-Bromo-6-deoxy-cellulose acetate, propionate, and butyrate esters exhibited solubility properties similar to one another

The ^{13}C NMR spectra for the 6-bromo-6-deoxy-cellulose esters are shown in Figures 3.4, 3.5, and 3.6. The chemical shift for *C*-6 substituted by bromine is seen in each spectrum at 32 ppm. The *C*-1 peak is found at 100 ppm, and the peaks for the remaining ring carbons (*C*-2, 3, 4, and 5) all fall between 70 and 80 ppm. For the 6-bromo-6-deoxy-2,3-di-*O*-acetyl-cellulose spectrum, the carbonyl carbon signals occur at 169 and 170 ppm, while the methyl carbon from the acetate is at 21 ppm. The carbonyl carbons for both the propionate and the butyrate ester derivatives are found at 172 and 173 ppm. The methyl and methylene groups from the propionate esters show up as peaks at 9 and 27 ppm respectively, while the methyl and two methylene groups from the butyrate esters occur at 13, 18, and 35 ppm, respectively. Small peaks at 21 (methyl), 62 (*C*-6), and 170 ppm (carbonyl) in the spectra for the propionate and butyrate ester derivatives indicate the presence of a low DS of acetate ester groups attached to *C*-6 of cellulose. The occurrence of these acetate groups was reported by Furuhata et al. (1992b) after a carbonyl absorption was noticed in the FTIR spectrum of brominated cellulose that had not been treated with any esterification reagents. They proposed that the acetate groups were a product of the DMAc solvent acting as a nucleophile instead of bromide during the S_N2 reaction with the cellulose alkoxyphosphonium salt intermediate. This would result in the formation of an iminium salt, which would then be hydrolyzed to the acetate upon aqueous work-up (Scheme 3.1).

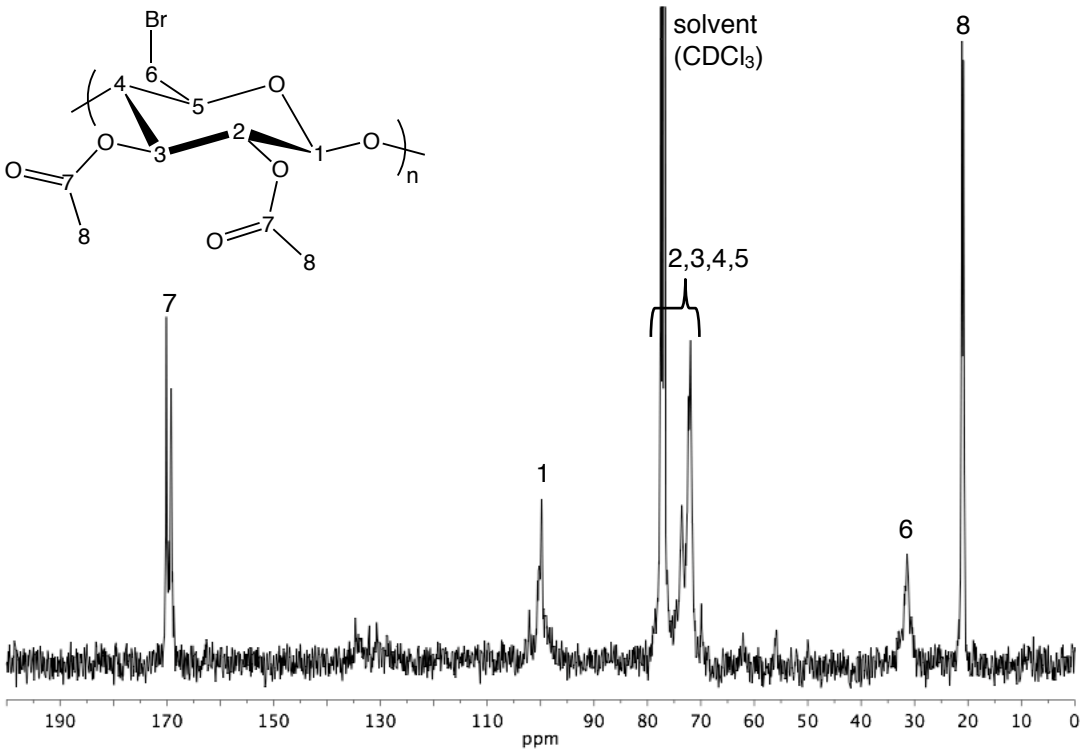


Figure 3.4 ^{13}C NMR spectrum of 6-bromo-6-deoxy-2,3-di-*O*-acetyl-cellulose

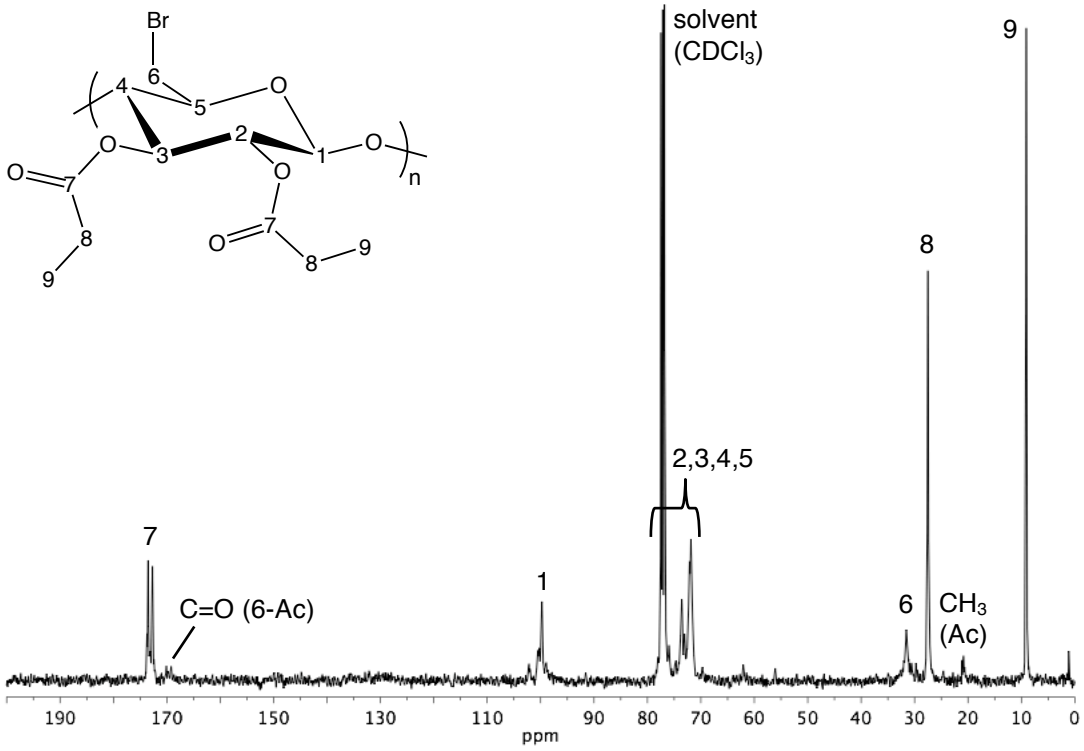


Figure 3.5 ^{13}C NMR spectrum of 6-bromo-6-deoxy-2,3-di-*O*-propionyl-cellulose

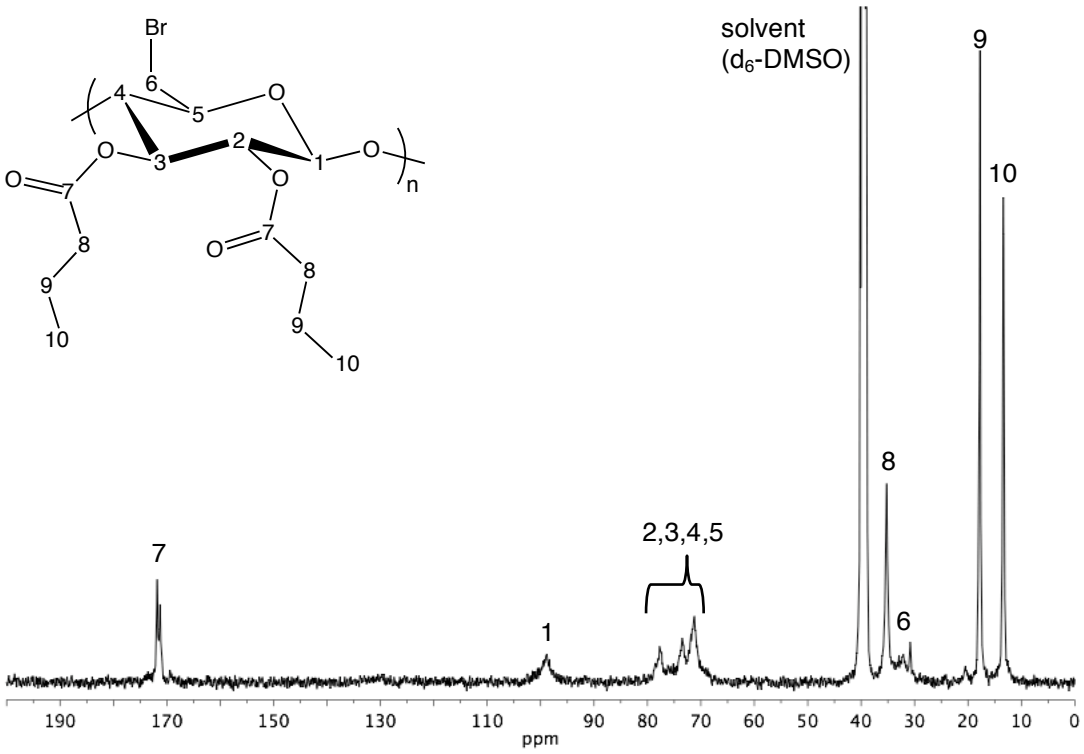
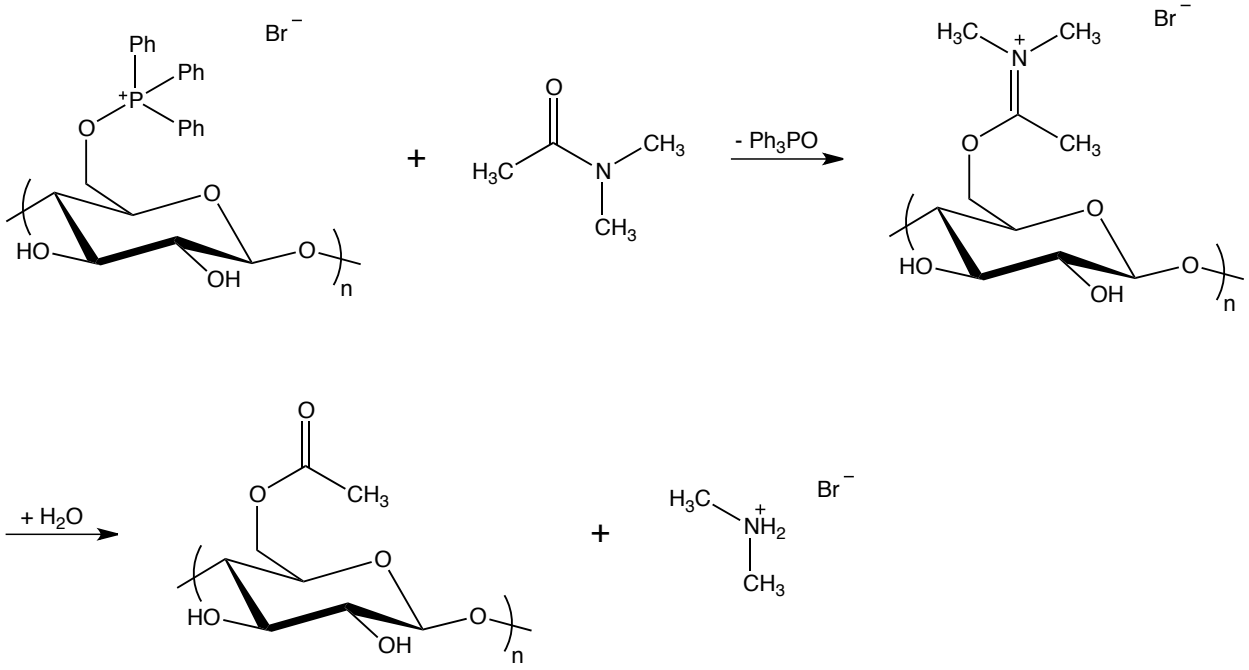


Figure 3.6 ^{13}C NMR spectrum of 6-bromo-6-deoxy-2,3-di-*O*-butyryl-cellulose



Scheme 3.1 The mechanism proposed by Furuhata et al. (1992b) for the side reaction during regioselective bromination in DMAc/LiBr that results in acetylation at *C*-6

The ^{1}H NMR spectrum for 6-bromo-6-deoxy-2,3-di-O-acetyl-cellulose is shown in Figure 3.7. The proton spectra for the propionyl and butyryl esters are nearly identical in the backbone proton region between 3.0 and 5.5 ppm, though they do show the additional aliphatic protons as expected. The overlapping signals for the hydrogen atoms in the backbone region make it difficult to accurately assign the peaks to each proton. This can be interpreted as evidence that the substitution on the cellulose derivatives does not occur with perfect regioselectivity, as a derivative with perfectly regioselective substitution could be expected to have more discrete peaks as is seen with cellulose triacetate (Heinze, et al. 2006). In this case, the presence of acetyl groups attached to *C*-6 may account for the overlapping peaks.

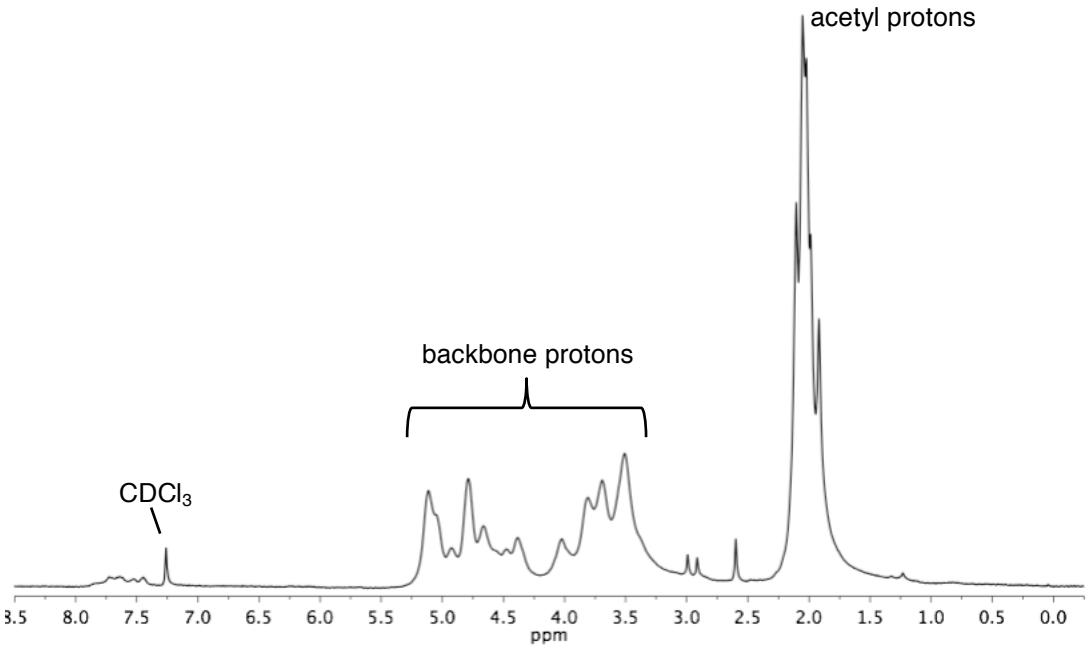


Figure 3.7 ^{1}H NMR spectrum for 6-bromo-6-deoxy-2,3-di-*O*-acetyl-cellulose

The bromide DS values, degrees of polymerization (DP), and isolated yields for the products of the bromination and esterification reactions are shown in Table 3.2. In each case, the DS of the bromide was determined to be near 1.0. In their original paper, Furuhata et al.

determined that the DS of their non-esterified product was 0.91 (Furuhata, et al. 1992b), and the highest DS obtained using this reaction and reported in the literature was 0.98 (Matsui, et al. 2005). Ours is the first literature report of a DS of greater than 1.0 from this reaction. However, especially considering the competing acetylation reaction at carbon 6 discussed in the previous paragraph, the possibility of substitution at secondary hydroxyl groups in the previous papers cannot be confirmed or ruled out on the basis of these near 1.0 DS numbers alone. The DP of the starting microcrystalline cellulose was determined to be 80 by carbanilation of the polymer with phenyl isocyanate followed by GPC analysis. The molecular weight data for the 6-bromo-6-deoxy-2,3-di-*O*-acyl-celluloses indicate that little or no polysaccharide chain degradation occurs during the reaction.

Table 3.2 Results from the one-pot regioselective bromination and esterification of microcrystalline cellulose

Acyl group	Bromide DS[1]	DP[2]	Yield
Acetyl	1.04	66	91%
Priopionyl	0.94	80	92%
Butyryl	1.10	84	89%

[1)] Determined by elemental analysis

[2)] Determined by GPC. The DP of the starting cellulose was 80.

Thermal properties of 6-bromo-6-deoxy-2,3-di-*O*-acyl-cellulose

The thermal properties of the 6-bromo-6-deoxy-2,3-di-*O*-acyl-celluloses were evaluated using TGA and DSC. Figure 3.8 shows a TGA thermogram of 6-bromo-6-deoxy-2,3-di-*O*-acetyl-cellulose that is typical for all of the samples tested. In each case, the samples were thermally stable up to around 210 to 215 °C, at which point they displayed an abrupt and dramatic weight loss equaling approximately 60% of the starting mass. We have not investigated the nature of this decomposition in detail.

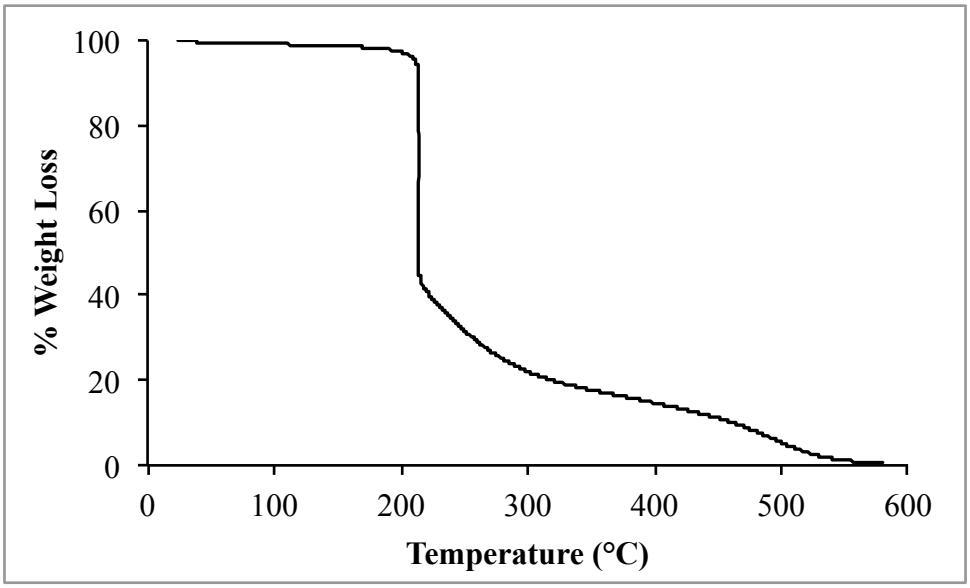


Figure 3.8 A TGA thermogram of 6-bromo-6-deoxy-2,3-di-*O*-acetyl-cellulose

DSC analysis was performed to determine transition temperatures of the materials. None of the samples displayed any crystalline melting endotherms or crystallization exotherms upon heating. A glass transition temperature (T_g, second heating scan) was only detected for 6-bromo-6-deoxy-2,3-di-*O*-butyryl-cellulose and not for the acetyl and propionyl esters (Figure 3.9). It is presumed that the T_g's for the 6-bromo-6-deoxy-cellulose acetate and propionate esters are above 180 °C, which was the maximum temperature of the experiments due to the decomposition just above 200 °C observed in the TGA spectra. For comparison cellulose triacetate, tripropionate, and tributyrate exhibit glass transitions around 172 °C, 133 °C, and 92 °C, respectively (Klarman, et al. 1969).

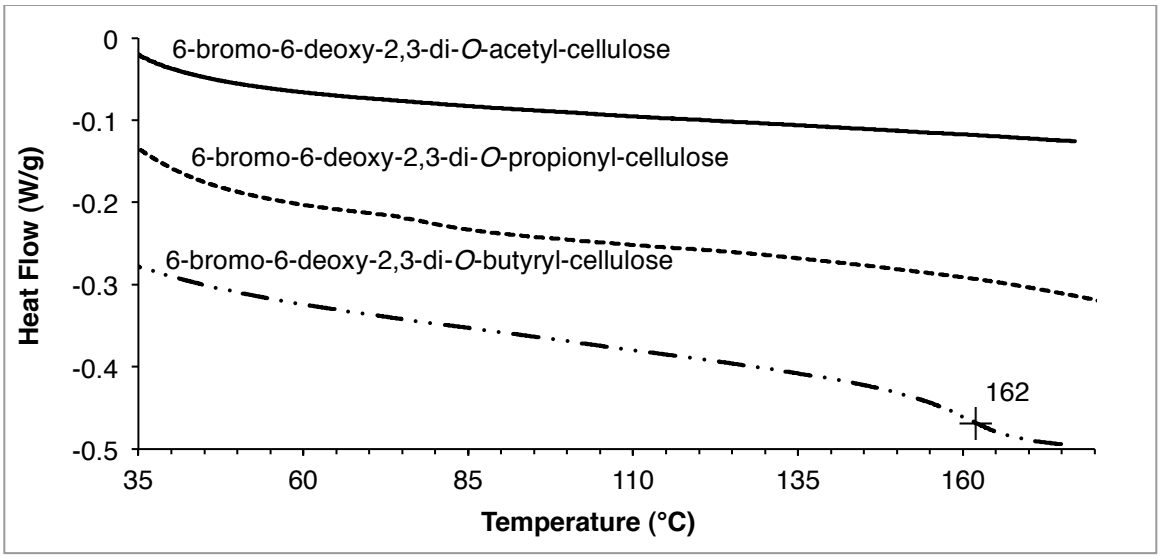


Figure 3.9 DSC thermograms for 6-bromo-6-deoxy-2,3-di-*O*-acyl-cellulose. (The y-axis values for the curves were offset for clarity.)

Conclusions

The solubility of cellulose regioselectively brominated at *C*-6 in organic solvents was drastically improved by esterification of the remaining free hydroxyl groups with acetic, propionic, or butyric anhydride. The acylation was performed without first isolating the brominated cellulose, allowing for the synthesis of 6-bromo-6-deoxy-2,3-di-*O*-acyl-celluloses in a one-pot reaction from microcrystalline cellulose. The chemical structures of the derivatives have been confirmed by FTIR and ^{13}C NMR spectroscopy as well as by elemental analysis. Molecular weight analysis indicates that the bromination and esterification reactions result in little degradation of the polysaccharide backbone. These derivatives are very promising precursors to other regioselectively substituted cellulose derivatives due to their good solubility, very good regioselectivity of substitution, and the ability of the bromide to act as a leaving group for further substitution reactions. Examples of such substitution reactions will be discussed in the following chapters.

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CHAPTER 4: ATTEMPTED SYNTHESIS OF REGIOSELECTIVELY CARBOXYLATED CELLULOSE DERIVATIVES BY CARBONYL INSERTION AND NITRILE HYDROLYSIS REACTIONS

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Abstract

Two different methods were explored in attempts to synthesize new regioselectively carboxylated cellulose derivatives. The first method involved the use of a rhodium carbonyl catalyst for a carbonyl insertion reaction on iodinated tosyl cellulose. Reaction conditions similar to those used in the industrial syntheses of acetic acid (the Monsanto process) and acetic anhydride (the Eastman Chemical Company process) were used in these attempts. However, ^{1}H NMR analysis of the products indicated that the cellulose backbone did not survive the reaction. The second method involved the synthesis of 6-cyano-6-deoxy-2,3-di-*O*-acyl-cellulose derivatives from 6-bromo-6-deoxy-2,3-di-*O*-acyl-cellulose, followed by the attempted alkaline hydrolysis of the nitrile group. Conversion of the brominated cellulose to the cellulose nitrile was successful, representing the first reported synthesis of regioselectively synthesized nitrile derivatives. However, the cellulose nitriles underwent rapid degradation under alkaline hydrolysis conditions, thwarting efforts to convert them to carboxylated cellulose derivatives.

Introduction

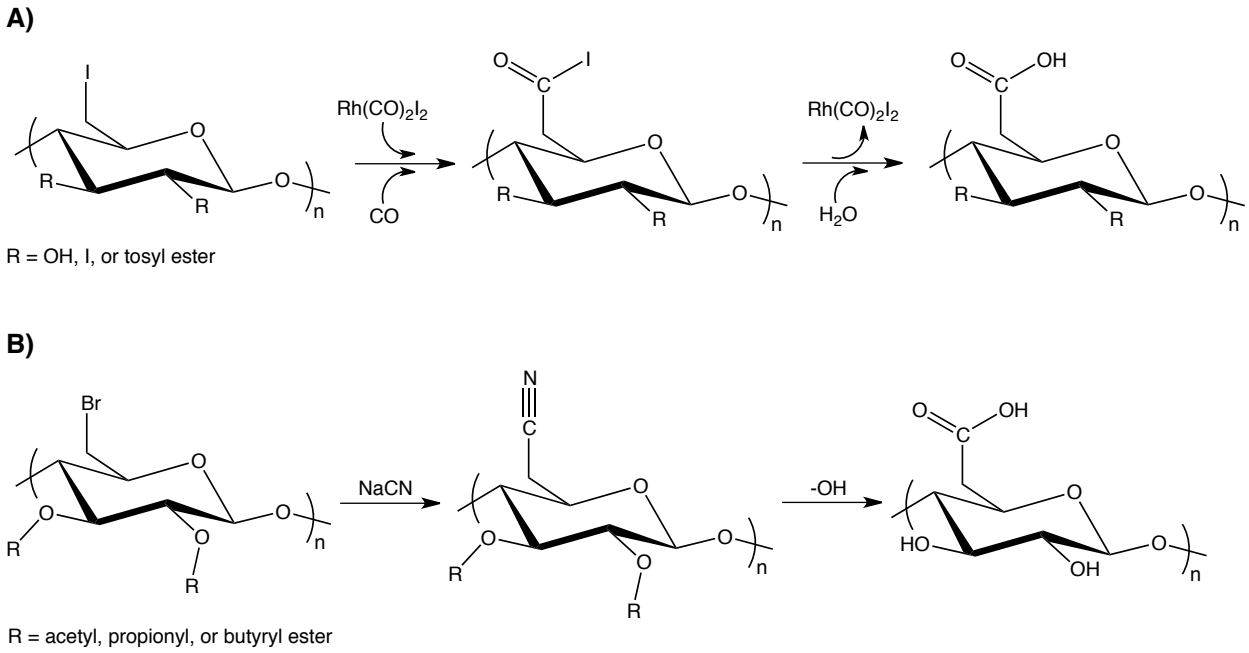
Once ingested, an orally administered drug must overcome several obstacles before it can have an intended therapeutic effect. Among these obstacles is the need for the drug to dissolve in the gastrointestinal fluids before it can be absorbed into the patient's bloodstream. This in particular currently presents a major challenge to the pharmaceutical industry. It has been estimated that 40% of currently marketed drugs along with about 60% of drugs in the

development pipeline have poor aqueous solubility (Fahr and Liu 2007). For many of these drugs, poor solubility has been identified as the primary obstacle to increasing the drug's bioavailability and efficacy.

One method that has been proven effective for improving aqueous solubility is the formation of a solid dispersions of a drug in an amorphous polymer matrix (Leuner and Dressman 2000). In ideal cases, these solid dispersions are similar to liquid solutions in that the drug is molecularly dispersed within the polymer, forming a single phase. The crystallinity of the drug is thus disrupted, eliminating the requirement for dissolution of overcoming its heat of fusion. Once the drug dispersion enters the body, gastrointestinal fluids must permeate the polymer matrix, which then swells or dissolves to allow the drug to diffuse out of the matrix. As a result, polymers used in this application must be both non-toxic and permeable to water. Additionally, it is highly beneficial if the polymer has a high glass transition temperature so that the mobility of the drug molecules within the matrix is restricted even under high humidity or high ambient temperatures, inhibiting potential recrystallization. Each of these characteristics is common to cellulose derivatives, making them good candidates as amorphous matrix polymers. In fact, several studies have shown that cellulose derivatives such as cellulose acetate phthalate (C-A-P), hydroxypropyl methylcellulose phthalate (HPMCP), hydroxypropyl methylcellulose acetate succinate (HPMCAS), and carboxymethylcellulose acetate butyrate (CMCAB) are effective polymers for forming solid dispersions with a number of different drugs (Edgar 2007; Friesen, et al. 2008; Konno, et al. 2008; Posey-Dowty, et al. 2007).

Our lab is interested in synthesizing new cellulose derivatives with chemical properties tailored for use with specific types of drugs in amorphous solid dispersion formulations. One of the primary challenges in effectively dispersing drug molecules in a polymer matrix is ensuring the miscibility of the drug with the polymer. Formulations without good miscibility are prone to phase separation, which may result in drug recrystallization over time (Qian, et al. 2010). Miscibility of the drug in the polymer can be improved by increasing specific interactions (e.g. hydrogen bonding, van der Waals, and ionic interactions) between the two materials (Marsac, et al. 2006b; Marsac, et al. 2006a; Marsac, et al. 2009). Thus, one of the goals of this research is to synthesize new cellulose derivatives with functional groups, such as amino or carboxyl groups, that are capable of strong interactions with different drugs.

Additionally, this research was also aimed at synthesizing new cellulose derivatives using regioselective substitution methods. A growing body of literature provides evidence that controlling the position of substitution on cellulose derivatives can lead to products with different solubility properties among derivatives with the same degree of substitution (DS) (Fox, et al. 2011). Given the assumption that the solubility properties of a cellulose derivative and its miscibility with drugs are related, it could be reasonably expected that regioselectively substituted cellulose derivatives would exhibit different miscibility with a given drug than would a corresponding randomly substituted cellulose derivative. However, there has been no published research exploring this hypothesis.



Scheme 4.1 The general reaction pathways investigated for the synthesis of regioselectively carboxylated cellulose. A) rhodium catalyzed carbonylation of 6-deoxy-6-iodo-cellulose derived from tosyl cellulose. B) hydrolysis of cellulose nitriles synthesized from 6-bromo-6-deoxy-cellulose esters.

The experiments described in this chapter were attempts to synthesize new regioselectively carboxylated cellulose derivatives in the hopes of investigating them for drug delivery applications. Two different approaches were used in these attempts. The first involved a

rhodium catalyzed carbonyl insertion reaction on 6-deoxy-6-halo-cellulose followed by aqueous hydrolysis of the resulting acid halide (Scheme 4.1, reaction A). The idea for attempting these reactions was inspired by the Monsanto and Eastman processes used to synthesize acetic acid and acetic anhydride from methanol and methyl acetate, respectively (Zoeller, et al. 1992; Haynes 2006). An alternative approach to the same products was attempted by synthesizing 6-cyano-6-deoxy-cellulose by substituting the halide on 6-deoxy-6-halo-cellulose with the cyanide anion. It was thought that alkaline hydrolysis of the nitrile group would afford the regioselectively carboxylated cellulose derivative (Scheme 4.1, reaction B). Both iodinated tosyl cellulose (Rahn, et al. 1996) and the 6-bromo-6-deoxy-cellulose esters detailed in Chapter 3 were used as the halogenated cellulose substrate in these reactions. This chapter will describe these synthetic reactions and discuss their results.

Experimental

Materials

Microcrystalline cellulose (MCC, Avicel® PH-101, Fluka) was dried under reduced pressure at 50 °C overnight prior to use. Lithium chloride (LiCl, Fisher), lithium bromide (LiBr, Fisher), sodium iodide (NaI, Fisher), and sodium cyanide (NaCN, Sigma) were dried under reduced pressure at 125 °C. N-Bromosuccinimide (NBS, Sigma) was recrystallized from boiling water and dried for two days under reduced pressure over anhydrous calcium chloride. Triethylamine, *p*-toluenesulfonyl chloride (Aldrich) triphenylphosphine (Ph_3P, Strem), lithium acetate (Sigma), dicarbonylacetylacetonato rhodium (I) (Strem), all carboxylic acid anhydrides (Acros), and phenyl isocyanate (Acros) were used as received. Dimethylacetamide (DMAc, Fisher) and dimethyl sulfoxide (DMSO, Acros) were kept over 4 Å molecular sieves and stored under dry nitrogen until use. Ethanol (Fisher), acetone (Fisher), methanol (Fisher), and 2,4-pentanedione (Alfa Aesar) were used as received.

Measurements

^{13}C NMR spectra were obtained on Varian INOVA or UNITY 400 MHz spectrometers with a minimum of 5,000 scans in either $CDCl_3$ or d_6-DMSO. Chemical shifts are reported

relative to the solvent peaks. A Thermo Electron Nicolet 8700 FTIR was used to perform infrared spectroscopic analysis of the samples as pressed KBr pellets. Molecular weight determination was achieved by gel permeation chromatography in N-methylpyrrolidone containing 0.05% lithium bromide using a Waters 1515 isocratic HPLC pump, Viscotek 270 dual detector, and Waters 2414 refractive index detector. Universal calibration curves were prepared using polystyrene standards. All elemental analyses were performed by Atlantic Microlab, Inc. Carbon, hydrogen, and nitrogen contents were determined using either Perkin Elmer 2400 II or Carlo Erba 1108 elemental analyzers. Bromine contents were determined by flask combustion followed by ion chromatography. Differential scanning calorimetry (DSC) was performed on a TA Instruments Q2000 DSC. Each run consisted of a 10 °C/min heating ramp from 30 °C to 200 °C, followed by a 10 °C/min cooling ramp back to 30 °C, and then a second heating ramp at 10 °C/min to 200 °C. Thermal transitions were recorded from the second heating cycle.

Dissolution of MCC in DMAc/LiCl

To a 1 L three-necked round-bottom flask, 20.0 g dried MCC (123.4 mmol anhydroglucose units (AGU)) was added. The flask was fitted with a nitrogen inlet, thermometer, and an overhead stirring adapter. Next, 470 mL DMAc was added to the flask, and the contents were stirred. The flask was flushed with dry nitrogen and heated in an oil bath to 160 °C. As the flask was heated, 40 mL of the DMAc was distilled from the flask. The slurry was kept at 160 °C for 1 h, after which it was allowed to cool to 90 °C. Next, 40 g LiCl (0.944 mol) was added to the flask and dissolved in the DMAc. The contents of the flask were allowed to cool to room temperature while being stirred continuously, and the MCC dissolved upon cooling to form a transparent solution. All cellulose solutions were kept under dry nitrogen until use within 24 h.

Tosylation of MCC

The temperature of a solution of MCC in DMAc/LiCl was lowered to 8 °C by placing the flask in a cooling bath. A mixture of 69 mL triethylamine (4 eq. per AGU) and 48 mL DMAc was added dropwise to the cellulose solution. Next, 47.03 g *p*-toluenesulfonyl chloride was dissolved in 70 mL DMAc, and the solution was added dropwise to the reaction flask. The reaction solution was kept under nitrogen and continuously stirred for 24 h at 8 °C, after which

the reaction was halted by pouring the solution into 3 L water. The precipitate that formed was filtered out and washed with an additional 2 L of water. The product was then soaked in 500 mL ethanol overnight and then filtered out once again. It was next redissolved in acetone and reprecipitated in 3 L water. The product was filtered out once again and dried in the vacuum oven.

Iodination of cellulose tosylate

To a 1 L three-necked round-bottom flask, 20.23 g tosyl cellulose was added. The tosyl cellulose was dissolved in 500 mL 2,4-pentanedione. The solution was stirred under nitrogen and heated to 130 °C, after which 33.7 g NaI was added to the solution. After 2 h, the reaction solution was added to 3.25 L ethanol, and the resulting precipitate was filtered out. Addition of the ethanolic filtrate to 4 L of water caused more product to precipitate, and this was filtered out as well. The collected product was soaked in a 0.1 M aqueous solution of sodium thiosulfate for two hours, and the product was filtered out again. It was washed with water, and then placed in a vacuum oven to dry.

Attempts at rhodium catalyzed carbonyl insertion on the iodinated cellulose

In a typical reaction, 0.500 g of the iodinated cellulose, 20 mg dicarbonylacetylacetonato rhodium (I) ($Rh(CO)_2Acac$), 0.230 g LiOAc, and 10 mL of a solvent were added to a 20 mL Parr high pressure reactor. The reactor was flushed with CO gas, sealed, and then heated to 180 °C. Once it reached the desired temperature, the reactor was pressurized to 750 psi with CO gas. After 2 h, the reactor was allowed to cool, and then it was depressurized. An appropriate antisolvent was used to precipitate the product, after which it was filtered out.

Dissolution of MCC in DMAc/LiBr

To a 1 L three-necked round-bottom flask, 5.00 dried MCC (30.8 mmol AGU) was added. The flask was fitted with a nitrogen inlet, thermometer, and an overhead stirring adapter. Next, 225 mL DMAc was added to the flask, and the contents were stirred. The flask was flushed with dry nitrogen and heated in an oil bath to 160 °C. As the flask was heated, 25 mL of the DMAc was distilled from the flask. The slurry was kept at 160 °C for 1 h, after which it was allowed to

cool to 90 °C. Next, LiBr (45 g, 0.518 mol) was added to the flask and dissolved in the DMAc. The contents of the flask were allowed to cool to room temperature while being stirred continuously. The MCC dissolved within 2 h to form a transparent solution. All cellulose solutions were kept under dry nitrogen until use within 24 h.

Synthesis of 6-bromo-6-deoxy-2,3-di-*O*-acyl-cellulose

Ph_3P (32.35 g, 4 eq per AGU) was dissolved in 100 mL dry DMAc. A second solution of NBS (21.95 g, 3 eq per AGU) was made in an additional 100 mL dry DMAc. The Ph_3P solution was added to the MCC solution dropwise via a liquid addition funnel. The NBS solution was added likewise after the addition of the Ph_3P solution. The reaction solution was then heated to 70 °C, and the reaction was allowed to continue for 1 h. Esterification of the remaining hydroxyl groups was accomplished by the dropwise addition of 5 eq of a carboxylic acid anhydride (acetic, propionoic, or butyric anhydride) followed by continued reaction overnight at 70 °C. The reaction was halted by allowing it to cool to room temperature and then adding it slowly to 4 L of a 50:50 mixture of methanol and deionized water, precipitating the product. The precipitate was recovered by filtration and then re-dissolved in acetone. The acetone solution was concentrated under reduced pressure, and the product was reprecipitated in ethanol. The isolated product was dissolved in acetone and reprecipitated in ethanol one more time, and then it was dried under reduced pressure at 50 °C.

Synthesis of 6-cyano-6-deoxy-2,3-di-*O*-acyl-cellulose

In a representative example, NaCN (1.2 eq per brominated AGU, ***CAUTION: NaCN is highly toxic and must be handled and disposed of safely! (Rubo, et al. 2000)***) was dissolved in 50 mL DMSO in a nitrogen flushed 250-mL round-bottom flask with a magnetic stir bar. The solution was heated to 50 °C, and 6-deoxy-6-bromo-2,3-di-O-acyl-cellulose (1.0 g) was added and dissolved. The reaction was continuously stirred for 4 h, and then it was cooled to room temperature. The product was precipitated in 450 mL deionized water and recovered by filtration. The precipitate was re-dissolved in 15 mL acetone and precipitated again in 100 mL deionized water followed by filtration. The product was dried in a vacuum at 50 °C.

Carbanilation of microcrystalline cellulose

Samples of the MCC used in the above experiments were derivatized according to a previously published procedure (Evans, et al. 1989) to prepare them for GPC analysis. Briefly, 100 mg dried MCC was suspended in 20 mL anhydrous pyridine. Then, 2 mL phenyl isocyanate was added to the flask. The mixture was heated under nitrogen in an oil bath at 80 °C for 24 h, during which time the MCC completely dissolved. To halt the reaction, 2 mL methanol was added. The solution was allowed to cool, and then it was added to 200 mL 30/70 methanol/water. The resulting precipitate was filtered out and thoroughly washed with additional 30/70 methanol/water. The product was then dried in a vacuum oven at 50 °C.

Results and Discussion

Synthesis of iodinated tosyl cellulose

Iodinated cellulose was synthesized using the procedure published by Rahn et al. (1996). In this procedure, tosyl cellulose is first synthesized by the homogeneous reaction of tosyl chloride with cellulose dissolved in DMAc/LiCl. The tosyl cellulose is then iodinated by reaction with sodium iodide in a ketone solvent (Finkelstein reaction conditions). The tosylation reaction has often been used in recent years as a method to regioselectively attach functional groups at carbon 6 (*C*-6, see Figure 2.1) on cellulose due to the fact that the tosyl chloride reacts preferentially with primary hydroxyl groups. The tosylate group can then serve as a good leaving group for S_N2 substitution reactions at the *C*-6 position. Rahn et al. (1996), described a method to conveniently measure the DS of the tosylated cellulose from the infrared spectrum of the material by determining the ratio of the peak intensities at 1176 cm^{-1} (tosylate) and 1065 cm^{-1} (cellulose backbone). This ratio can then be referenced to a calibration curve published in their paper that relates it to the DS of the derivative (originally determined by sulfur content). For the tosylated cellulose used in this research, the ratio of peak intensities at 1176 and 1065 cm^{-1} is 0.482/0.306 = 1.58 (Figure 4.1). This corresponds to a tosyl DS of about 1.3. Information about the distribution of the tosyl substituents at the different carbons on the AGU repeating unit of cellulose can be obtained from the ^{13}C NMR spectrum of the material (Figure 4.2). The signals for the carbons on the tosylate substituents are found between 125 and 150 ppm (aromatic ring

carbons) and at 21 ppm (methyl carbon). The cellulose backbone resonances are found between 58 and 103 ppm. The peaks at 60 and 68 ppm are assigned to the *C*-6 position and represent the unsubstituted and tosylated carbons, respectively. The carbon 1 (*C*-1) resonance is also split between 98 and 101 ppm due to tosyl substitution at carbon 2 (*C*-2) and unsubstituted *C*-2, respectively. Thus, it can be seen that tosylation does not occur exclusively at the *C*-6 position.

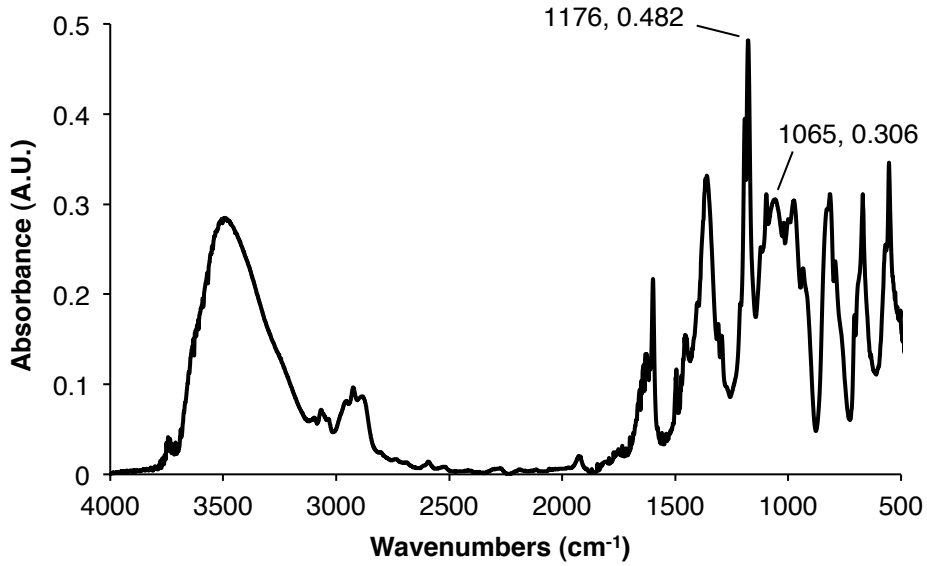


Figure 4.1 FTIR spectrum of tosylated cellulose

Iodination of tosylated cellulose has historically been utilized as a method for gaining further insight into the positions at which the cellulose was substituted. The assumption behind the analysis was that reaction of the tosylated cellulose with NaI under Finkelstein reaction conditions would result in the iodide displacing the tosylates exclusively at *C*-6. Thus, based on the iodine and residual sulfur content of the product, the tosyl DS of the starting material at *C*-6 relative to that at *C*-2 and *C*-3 could be calculated. Using this method, Rahn et al. (1996) estimated that cellulose tosylated in DMAc/LiCl needed to reach a DS of 1.4 before all the *C*-6 positions were substituted (see Figure 2.3). However, ^{13}C NMR analysis of iodinated tosyl cellulose shows that iodide substitution also occurs at *C*-2, as seen in the splitting of the *C*-1 resonance into three peaks due to different substitution at *C*-2 (Figure 4.3). The resonance for *C*-

1 adjacent to an unsubstituted *C*-2 occurs at 101 ppm, while the resonances for *C*-1 adjacent to a *C*-2 substituted with a tosylate group or iodide occur at 98 or 104 ppm, respectively.

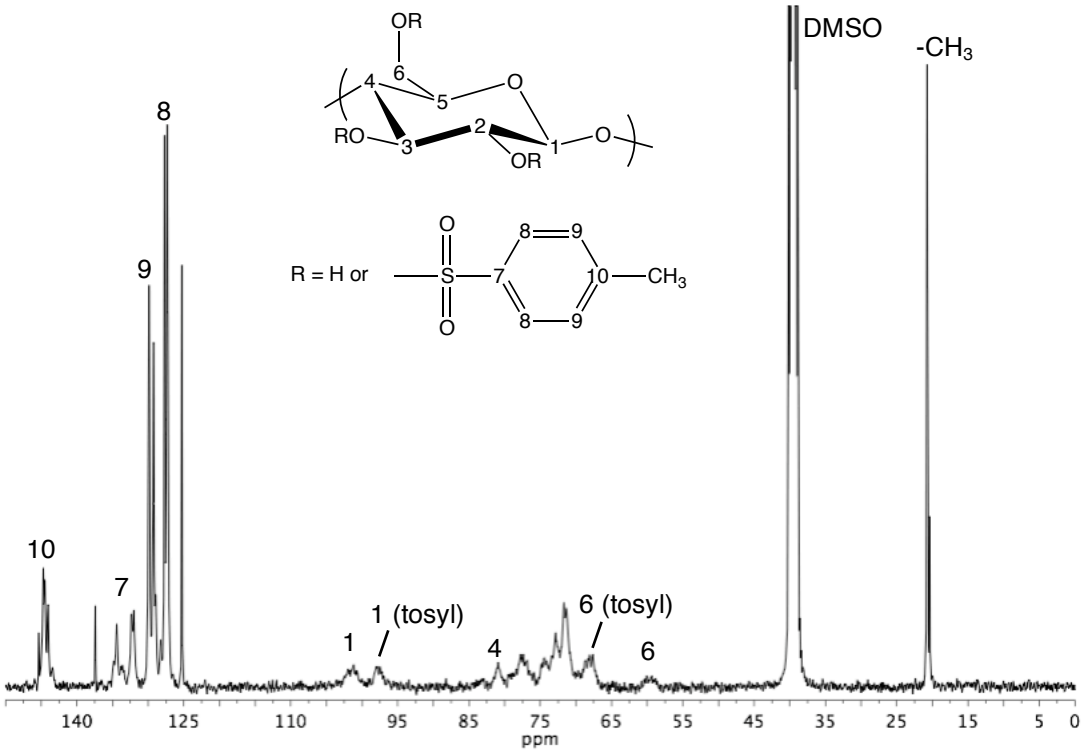


Figure 4.2 ^{13}C NMR of tosyl cellulose

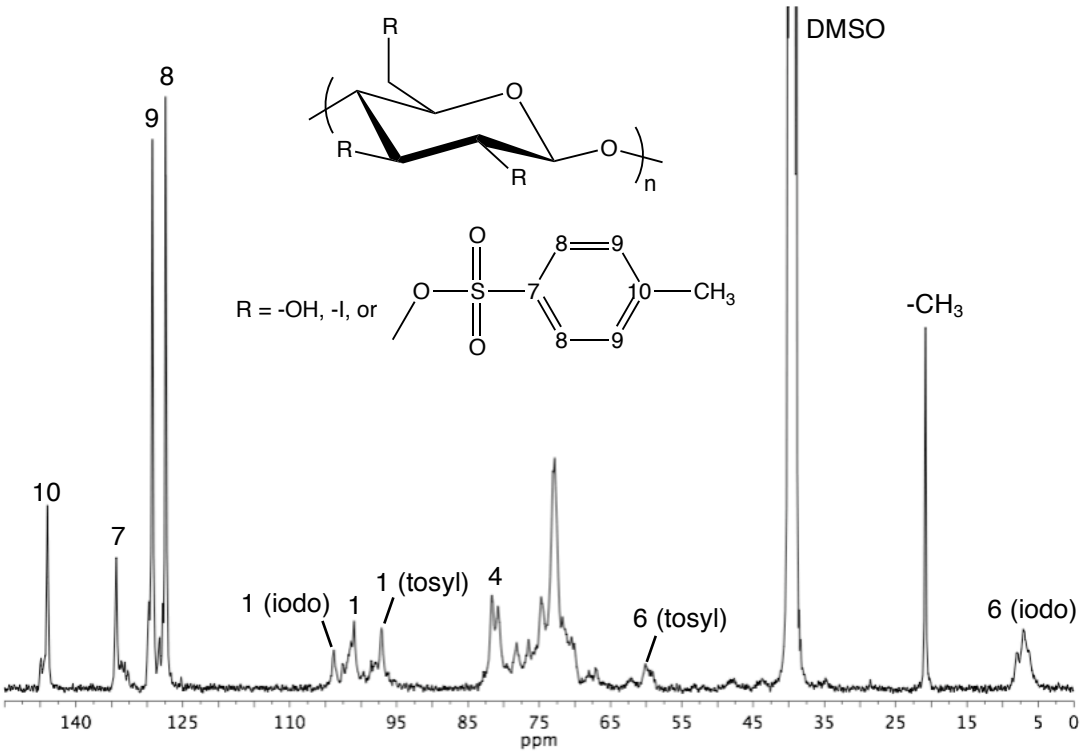


Figure 4.3 ^{13}C NMR of iodinated tosyl cellulose

For the carbonylation experiments attempted here, iodinated tosyl cellulose was synthesized to be used as a substrate. The iodinated *C*-6 resonance in the ^{13}C NMR spectrum of the material is located at 7 ppm (Figure 4.3). Some residual tosylated *C*-6 is apparent at 68 ppm. Estimation of the tosyl DS for the product using FTIR as described above gives a value of about 0.75. Since the tosyl DS for the starting material for the iodination reaction was about 1.3, the iodide DS can be calculated at 1.3 – 0.75 = 0.55 (Figure 4.4). However, this value is questionable since the tosylate groups at *C*-6 appear to be largely displaced, as evidenced by the ^{13}C NMR spectrum. Additionally, this reaction was performed under identical conditions to those reported by Rahn et al. (1996), who reported an iodide DS of 0.77 after a reaction with a starting tosyl cellulose with a DS of 1.32, as determined by elemental analysis. Thus, elemental analysis would appear to be a more accurate method for determining iodide DS, though it was not performed in this case. It is readily apparent, however, that the iodinated tosyl cellulose synthesized for this work has a significant iodide content, thought to be adequate for the subsequent carbonyl insertion experiments.

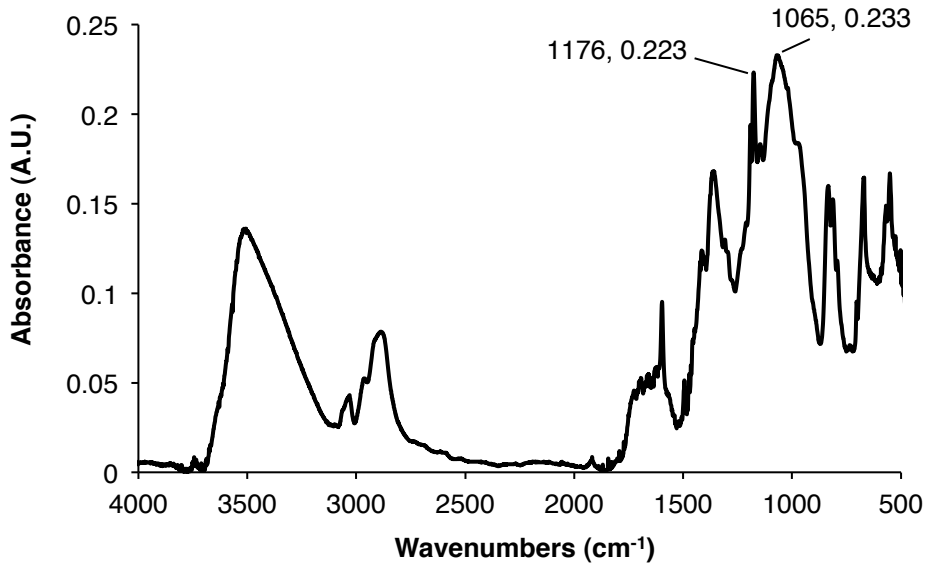
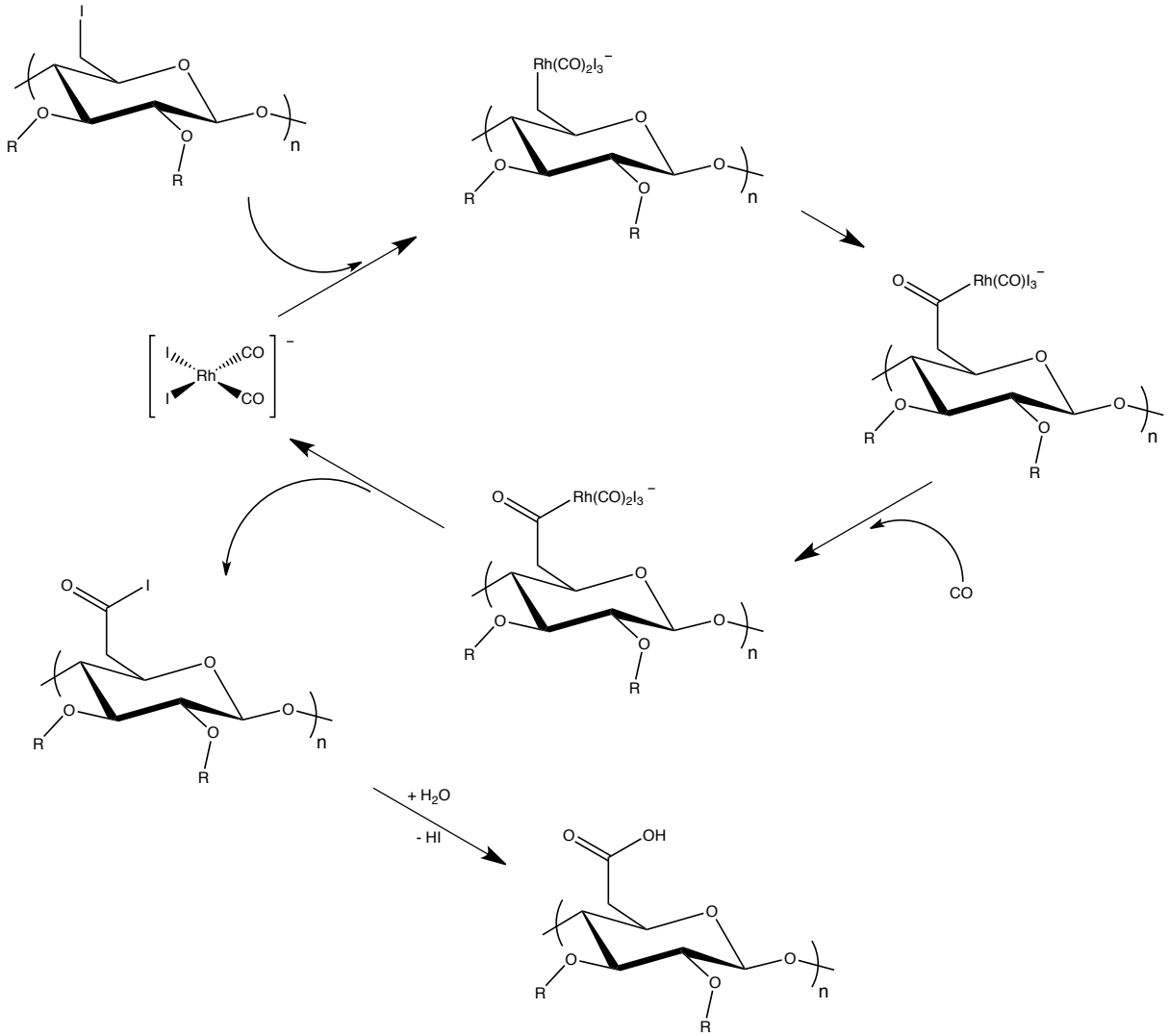


Figure 4.4 FTIR spectrum of iodinated tosyl cellulose

Attempted rhodium catalyzed carbonyl insertion with iodinated tosyl cellulose

The proposed reaction pathway for the rhodium catalyzed carbonyl insertion into the C-I bond of iodinated tosyl cellulose, including the rhodium catalytic cycle, is shown in Scheme 4.2. The reaction begins with the oxidative addition of the catalytic rhodium species $Rh(CO)_2I_2$ to the cellulose substrate. The cellulose would then migrate to one of the carbonyl ligands, followed by the addition of a new carbonyl ligand to the rhodium. The cellulose/rhodium complex next would undergo reductive elimination, resulting in a new acyl iodide group attached to cellulose and regenerating $Rh(CO)_2I_2$. The acyl iodide would be readily hydrolyzed to the carboxylic acid to generate the final product.



Scheme 4.2 The proposed reaction pathway for the rhodium catalyzed carbonyl insertion on iodinated tosyl cellulose

The reaction conditions chosen for the carbonyl insertion in these experiments, including the temperature, pressure, and relative quantities of reagents, were intended to mimic those described by Zoeller et al. (1992). Acetone, DMAc, and DMSO were all used as reaction solvents, each of which was capable of dissolving the substituted cellulose starting material and the rhodium catalyst precursor ($Rh(CO)_2Acac$). In each case, a brown liquid was recovered from the reaction vessel. The liquid was completely miscible in ethanol and DMSO, but a solid precipitate formed upon addition to water or dichloromethane. ^1H NMR spectra of the precipitated reaction products redissolved in d_6-DMSO showed no signals from protons on the

cellulose backbone, indicating that the cellulose had degraded. The chemical nature of the product was not explored further. One potential explanation for the apparent degradation of the cellulose backbone during this reaction could be that the rhodium/cellulose complex undergoes *β*-hydride elimination after the oxidative addition step of the catalytic cycle depicted in Scheme 4.2. When performing rhodium catalyzed carbonyl insertion on n-propanol, Dekleva and Forster (1985) found that the product was a mixture of n-butyric acid and isobutyric acid. They proposed that this was due to dissociation of CO ligand from the rhodium complex prior to the trapping of the rhodium-acyl species by the addition of another CO ligand. The dissociation of the CO ligand would result in a coordinatively unsaturated rhodium center, which could then undergo *β*-hydride elimination with the substrate. In the case of a cellulose derivative, *β*-hydride elimination could cause a chain reaction that resulted in the disintegration of the polysaccharide structure, especially at high temperatures.

Displacement of bromide by cyanide to synthesize 6-cyano-6-deoxycellulose derivatives

After the failure of the rhodium catalyzed carbonyl insertion reaction on iodinated tosyl cellulose, an alternative approach to regioselectively carbonylated cellulose was proposed. In this approach, cellulose that had been halogenated at *C*-6 was reacted with NaCN to form 6-cyano-6-deoxycellulose derivatives. Since the conversion of nitrile functional group to a carboxylic acid by acid or base hydrolysis is a well-known reaction in synthetic organic chemistry, it was thought that these new cellulose derivatives could be treated with a base to afford the desired carbonylated cellulose (Scheme 4.1, reaction B). Additionally, by the time these experiments were undertaken, my attempts to synthesize a more regioselective 6-deoxy-6-halo-cellulose than was possible via cellulose tosylates had yielded the results described in the previous chapter. Because of the higher regioselectivity of the 6-bromo-6-deoxy-cellulose esters, they were used as the substrates for synthesis of the 6-cyano-6-deoxy-cellulose derivatives.

Regioselectively synthesized 6-cyano-6-deoxycellulose esters were produced by the reaction of sodium cyanide with 6-bromo-6-deoxy-2,3-di-*O*-acyl-celluloses in DMSO. The ^{13}C NMR spectrum of 6-cyano-6-deoxy-2,3-di-*O*-propionyl-cellulose is shown in Figure 4.5. The peak for the 6-carbon has been shifted to 19.5 ppm, and the signal for the bromide substituted carbon at 32 ppm has nearly disappeared. Additionally, a new peak for the nitrile carbon appears at 117 ppm. The FTIR spectrum shows a weak absorption for the nitrile at 2255 cm^{-1} (Figure 4.6).

The DS of the nitrile group after 1 h of reaction at 50 °C is 0.40, and it increases to between 0.60 and 0.68 after 4 h (Table 4.1). Based on the elemental analysis data, a significant quantity of bromide remains despite the near disappearance of the 32 ppm 6-bromo peak in the ^{13}C NMR spectrum; the discrepancy is likely due to insufficient resolution in the ^{13}C NMR spectrum. The sum of the DS of the nitrile and the residual bromide approximately equal the DS of the bromide in the starting material, indicating that nucleophilic displacement of bromide by cyanide is the primary reaction observed.

However, molecular weight analysis of 6-cyano-6-deoxy-2,3-di-*O*-acetyl-cellulose isolated from the synthesis reaction after different lengths of time shows that the reaction results in some degradation of the polymer. The product isolated after 1 h of reaction with sodium cyanide had a DP of 54, almost 20% less than that of the starting material (Table 4.1). After 4 h of reaction, the DP of the 6-cyano-6-deoxy-2,3-di-*O*-acetyl-cellulose has decreased further to 32. Accordingly, the isolated yield from the reaction also decreases as the reaction progresses. While a 78% yield could be obtained after 4h at 50 °C, a reaction run at 70 °C for 2 h afforded a substantially reduced yield of 49%.

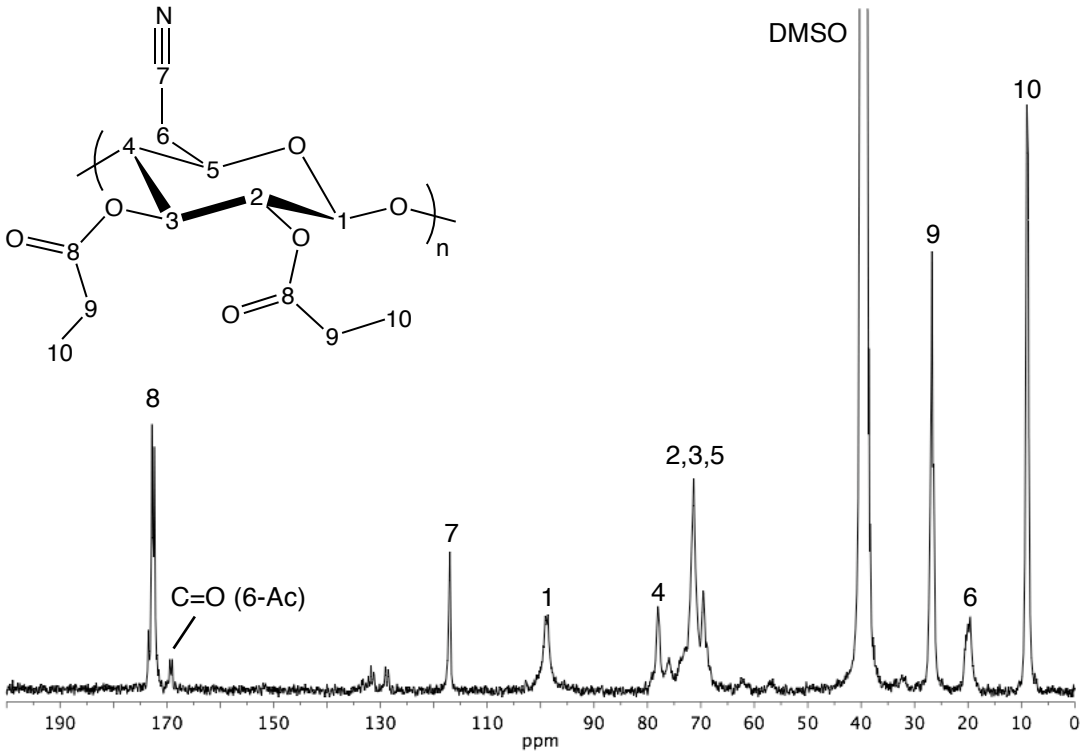


Figure 4.5 ^{13}C NMR spectrum for 6-cyano-6-deoxy-2,3-di-*O*-propionyl-cellulose

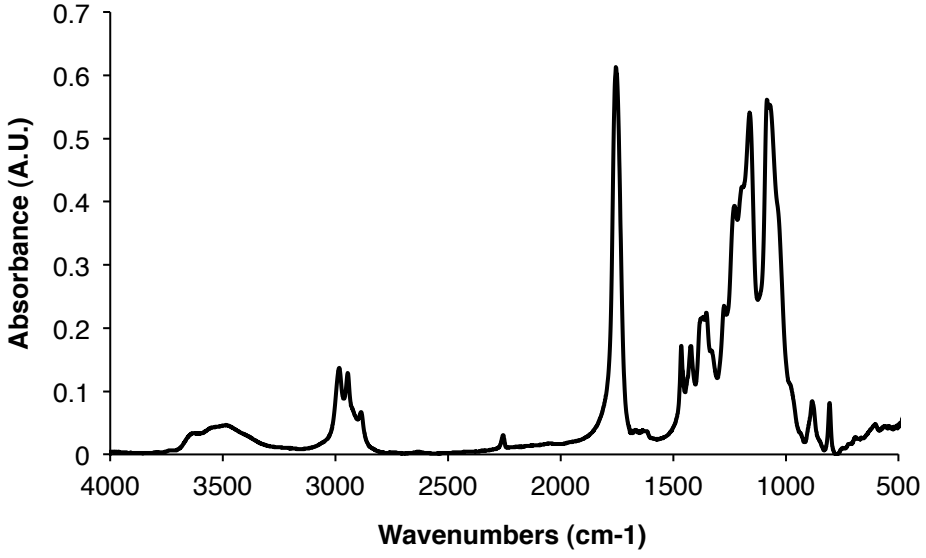


Figure 4.6 FTIR spectrum for 6-cyano-6-deoxy-2,3-di-*O*-propionyl-cellulose

Table 4.1 Results from the reaction of the 6-bromo-6-deoxy-cellulose esters with sodium cyanide in DMSO at 40 °C

		DS[1]				
Acyl group	Reaction Time (h)	CN	Br	Total	DP[2]	Yield
Acetyl	0	0	1.04	1.04	66	--
Acetyl	1	0.40	0.58	0.98	54	87%
Acetyl	2	0.51	0.44	0.95	58	85%
Acetyl	4	0.62	0.32	0.94	32	78%
Propionyl	4	0.60	0.28	0.88	45	80%
Butyryl	4	0.68	0.40	1.07	37	70%

[1)] Determined by elemental analysis

[2)] Determined by GPC

The thermal properties of the cellulose nitriles were investigated using DSC. None of the samples displayed any crystalline melting endotherms or crystallization exotherms upon heating, though glass transition temperatures (T_g, second heating scan) were detected for all of the 6-cyano-6-deoxy-cellulose esters (Figure 4.7). As expected, the glass transition temperature of the 6-cyano-6-deoxycellulose ester decreases as the length of the acyl chain increases from one carbon to three carbons. For each 2,3-diester, the T_g of the 6-cyano-6-deoxy cellulose derivative was lower than that of the corresponding 6-bromo-6-deoxy-cellulose derivative (Table 4.2)

Table 4.2 T_g values for the 6-bromo- and 6-cyano-6-deoxy-cellulose esters

C-6 substituent	C-2,3 substituent	T_g (°C)
Br	acetyl	> 200
Br	propionyl	> 200
Br	butyryl	162
CN	acetyl	188
CN	propionyl	164
CN	butyryl	147

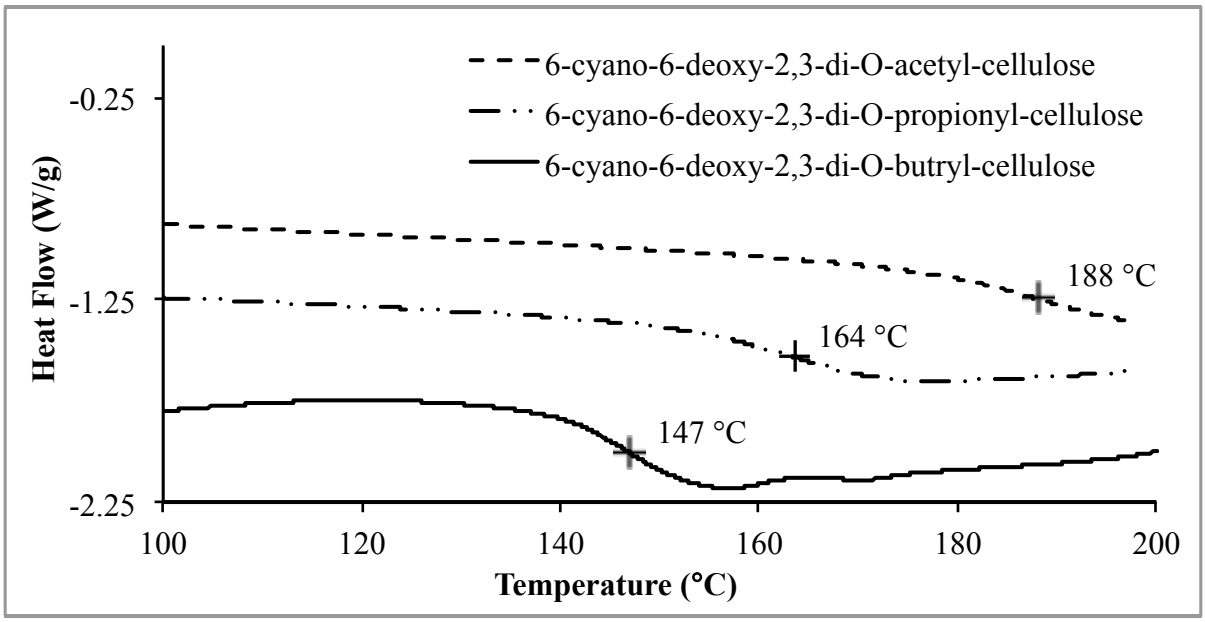
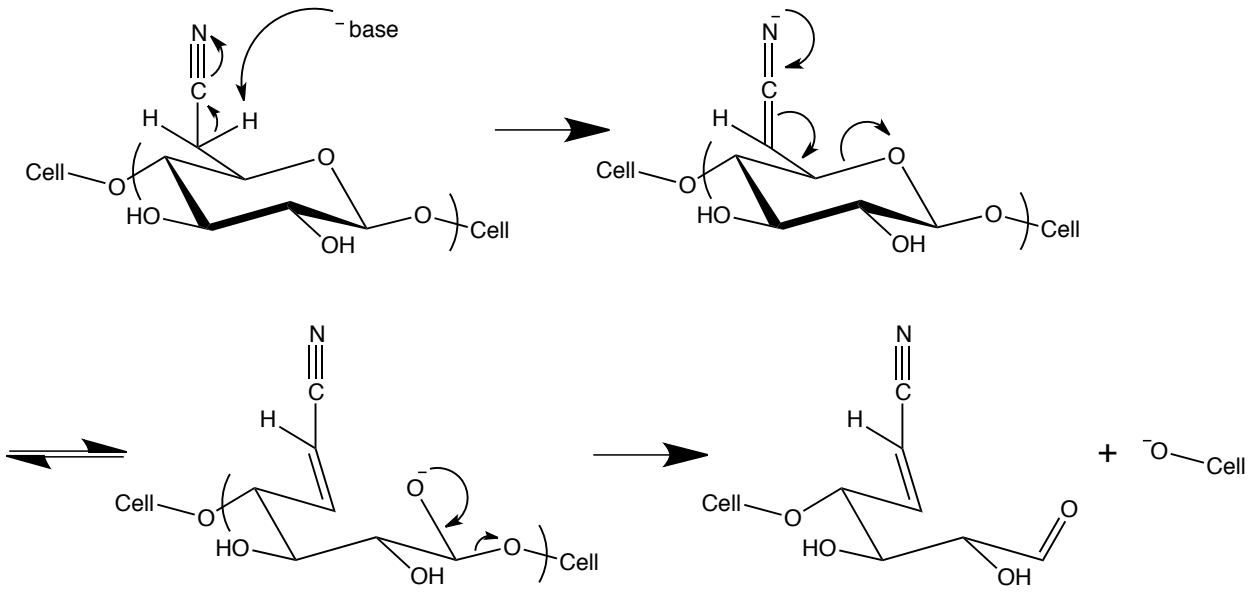


Figure 4.7 DSC thermograms (2nd heating scan) for the 6-cyano-6-deoxy-cellulose esters

Attempted hydrolysis and reduction of the nitrile groups

Acid or base catalyzed hydrolyses of nitriles to carboxylic acids are well-known reactions in organic chemistry. In our case, a regioselectively carboxylated cellulose derivative could have interesting properties for pH-controlled release and amorphous matrix formulations in oral drug delivery. Carboxylated cellulose derivatives synthesized through non-regioselective, conventional reactions are already widely used in oral drug formulations (Edgar 2007). 6-Carboxy-6-deoxycellulose derivatives would have the carboxyl tethered to the main chain by a one-carbon tether, thus would be unnatural analogs of cellouronic acid, obtained by TEMPO mediated oxidation of cellulose (Tahiri and Vignon 2000). Since the harsh reaction conditions necessary to hydrolyze nitriles at low pH would likely result in the degradation of the polymer backbone, several attempts were made to convert the nitrile groups to carboxylic acid groups under alkaline conditions. However, after many experiments, it was determined that the 6-cyano-6-deoxy-2,3-di-*O*-acyl-celluloses were highly prone to alkaline degradation. Even at room temperature, stirring the cellulose 6-cyano-6-deoxy-2,3-di-*O*-propionyl-cellulose in 8% aqueous sodium hydroxide results in a homogeneous solution after 1 h. Attempts to isolate any high molecular weight product by dialysis using a 3,500 MWCO membrane were not successful, with no product recovered. Attempts to hydrolyze the nitrile using hydrogen peroxide under very mild

alkaline conditions (McIsaac, et al. 1971; Katritzky, et al. 1989) returned the starting material. A possible mechanism for the alkaline degradation of the cellulose nitriles is shown in Scheme 4.3.



Scheme 4.3 Possible alkaline degradation mechanism for 6-cyano-6-deoxy-cellulose

Conclusions

Attempts were made to synthesize regioselectively carboxylated cellulose via two different methods. The first method involved a rhodium catalyzed carbonyl insertion reaction on iodinated tosyl cellulose. This reaction resulted in the complete degradation of the cellulose backbone, as determined by ^{1}H NMR. In the second method, 6-cyano-6-deoxy-cellulose esters were synthesized by reacting 6-bromo-6-deoxy-cellulose esters with NaCN in DMSO. This reaction proceeded smoothly, though molecular weight analysis of the products suggested that some degradation of the polysaccharide chain had occurred. The 6-cyano-6-deoxy-cellulose esters were found to be highly susceptible to alkaline degradation, thus preventing effective hydrolysis of the nitrile groups to produce carboxylic acids.

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CHAPTER 5: SYNTHESIS OF SELECTIVELY *O*-ACYLATED 6-AMINO-6-DEOXY-CELLULOSE

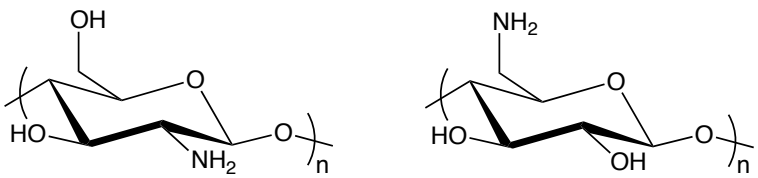
Abstract

Aminated polysaccharides have been extensively investigated for a wide range of biomedical applications. In many such applications, it can be beneficial to selectively modify the hydroxyl groups on the polysaccharide while leaving the amino groups unmodified. However, there are few good approaches to do this because of the similar reactivities of hydroxyl groups and primary amines. This research presents a new method that can produce aminated polysaccharides that are *O*-acylated with very high selectivity. The procedure involves the synthesis of 6-azido-6-deoxy-cellulose esters from 6-bromo-6-deoxy-cellulose esters. The azide groups are then selectively and mildly reduced using the Staudinger reaction to produce 6-amino-6-deoxy-2,3-di-*O*-acyl-cellulose derivatives. This procedure also offers a new method to regioselectively acylate cellulose derivatives with a different acyl substituent attached to *C*-6 through an amide bond than the acyl substituents attached at *C*-2 and *C*-3 through ester bonds.

Introduction

Past research has demonstrated that polysaccharides containing amine functional groups have properties beneficial to a number of pharmaceutical and biomedical applications. Chitosan in particular has been extensively studied in drug and gene delivery formulations due to its ability to electrostatically bind and encapsulate anionic compounds, such as nucleic acids and certain proteins, protecting them from degradative enzymes prevalent in the body (Thanou and Junginger 2005; Dash, et al. 2011; Rudzinski and Aminabhavi 2010; Mao, et al. 2010; Lai and Lin 2009; Kumar, et al. 2004). Chitosan also possesses mucoadhesive properties and promotes the opening of tight junctions between epithelial cells, enhancing paracellular transport of drug molecules through biological membranes (Grabovac, et al. 2005; Ranaldi, et al. 2002; Lehr, et al. 1992). These properties have been attributed to the presence of amino groups on the polysaccharide backbone that can interact with anionic molecules found in mucus and on cell surfaces.

In attempts to mimic and improve on the properties of chitosan for drug and gene delivery, many researchers have artificially modified neutral polysaccharides to incorporate amino groups. The most common method to achieve this has been to attach amine containing side chains to a polysaccharide backbone. Polysaccharides that have been modified in this manner include dextran (Sun, et al. 2008; Yudovin-Farber and Domb 2007; Eliyahu, et al. 2006), pullulan (Thakor, et al. 2009; Kanatani, et al. 2006; Jo, et al. 2007; Thomsen, et al. 2011), schizophyllan (Nagasaki, et al. 2004), curdlan (Ikeda, et al. 2007), and cellulose (Song, et al. 2010). Each of these polysaccharide derivatives was found to effectively encapsulate DNA and improve its transfection efficiency into model cells compared to naked DNA. Another approach, though less common, to artificially introduce amino groups to polysaccharides is by directly substituting hydroxyl groups along the polysaccharide backbone with amines. This can be accomplished via a multi-step synthesis by tosylating or halogenating the polysaccharide, reacting the resulting intermediate compound with an azide salt, and then reducing the azide to an amine. One particular benefit to this approach is that the reactions predominantly occur at primary hydroxyl group sites, providing a mechanism for regioselective derivatization of the polysaccharide (Fox, et al. 2011). This azide substitution and reduction pathway has been used to synthesize both 6-amino-6-deoxy-chitosan (Furuhata, et al. 1998; Satoh, et al. 2006) and 6-amino-6-deoxy-cellulose (Teshirogi, et al. 1979; Daly and Lee 1991; Liu and Baumann 2002; Matsui, et al. 2005; Heinze, et al. 2006a). The former compound was found to have *in vitro* gene carrier properties superior to chitosan, while the latter compound has been studied as a precursor to artificial heparin-like polymers in attempts to mimic that compound's anticoagulant properties (Teshirogi, et al. 1981; Baumann, et al. 2003). 6-Amino-6-deoxy-cellulose is also of interest since it is a structural isomer of fully deacetylated chitosan (Figure 5.1). However, despite this structural similarity, it has not yet been evaluated for drug or nucleic acid delivery applications.

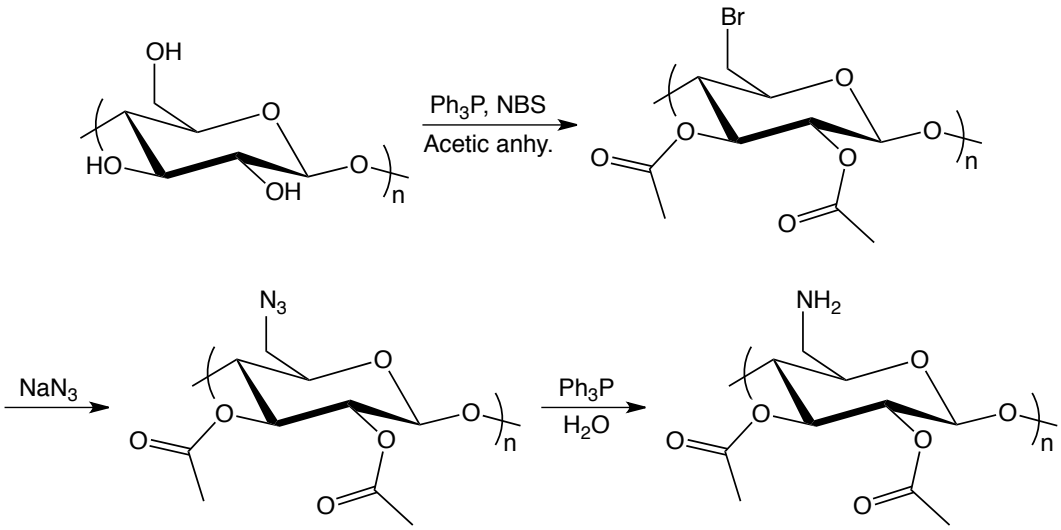


Chitosan (fully deacetylated) 6-amino-6-deoxy-cellulose

Figure 5.1 Chemical structures of chitosan and 6-amino-6-deoxy-cellulose

Polysaccharide derivatives intended for use in biomedical applications are often further modified to include additional chemical moieties other than amines that may also perform important functions. For example, additional derivatization of the polysaccharide can be used to alter its solubility in aqueous or organic solvents, attach a ligand for a specifically targeted cell membrane, or improve the affinity between the polymer and an encapsulated compound. These additional modifications are often achieved through ester linkages, due both to the prevalence of hydroxyl groups along the polysaccharide backbone and to the relatively mild conditions necessary for esterification. For polysaccharides with both amino and hydroxyl groups present, the problem then arises of finding selective substitution strategies that will result in reactions that modify the hydroxyls while not modifying the amino groups. The *N*-phthaloyl protecting group has been successfully demonstrated on chitosan to allow selective substitution at only the hydroxyl groups (Satoh, et al. 2006; Kurita, et al. 2001; Ifuku, et al. 2011). However, reduction with hydrazine is necessary to regenerate the free amine from the phthalimide, which would result in the cleavage of esters or other easily removable groups attached at the hydroxyl sites and could cause a reduction in the polysaccharide molecular weight as well. Chitosan can be preferentially *O*-acetylated by using acetyl chloride in methanesulfonic acid, though this procedure is not completely selective and results in significant degradation of the polysaccharide chain (Sashiwa, et al. 2002). An alternative approach that has only been briefly explored for the synthesis of *O*-acylated amino polysaccharides is to acylate an azide containing polysaccharide prior to reduction of the azide. In this approach, the azide would effectively act as a latent protected amine. The problem then becomes finding a reducing agent that can selectively convert the azide to the amine but not cleave the ester groups. To date, most efforts to convert azide groups attached to polysaccharides into amines have relied on lithium aluminum hydride (Liu and Baumann 2002; Teshirogi, et al. 1979) and sodium borohydride (Furuhata, et al. 1998; Matsui, et al. 2005; Heinze, et al. 2006a) as reducing agents, both of which are known to react with a variety of reducible functional groups including esters. Daly and Lee (1991) circumvented this problem by using 1,3-propanedithiol to selectively reduce the azide moieties on 6-azido-6-deoxy-cellulose esters to amines and retain the ester functionalities. However, while their method did result in the desired selectivity, only a small portion of the azide groups were reduced after 48 h of reaction, and only a very low free amine content was attainable.

In this chapter, the successful synthesis of 6-azido-6-deoxy-2,3-di-*O*-acyl-cellulose and its highly selective reduction to 6-amino-6-deoxy-2,3-di-*O*-acyl-cellulose under Staudinger reaction conditions is reported. The reaction scheme, shown in Scheme 5.1, begins with the synthesis of 6-bromo-6-deoxy-2,3-di-*O*-acyl-cellulose as described in the preceding chapters. This allowed the synthesis of 6-azido-6-deoxy-2,3-di-*O*-acetyl-cellulose by displacement of the bromide with an azide under completely homogeneous reaction conditions. The azide was then converted to an amine using the Staudinger reduction (Gololobov, et al. 1981). To our knowledge, this is the first demonstrated use of the Staudinger reduction on a polysaccharide substrate, and it is the first synthesis of an aminated polysaccharide with a high amino DS and completely selective *O*-acylation.



Scheme 5.1 The reaction scheme for the conversion of cellulose to 6-amino-6-deoxy-2,3-di-*O*-acetyl-cellulose

Materials and Methods

Materials

Microcrystalline cellulose (MCC, Avicel® PH-101, Fluka) was dried under vacuum at 50 °C overnight prior to use. Lithium bromide (LiBr, Fisher) and sodium azide (NaN_3, Acros) were dried under vacuum at 125 °C. N-Bromosuccinimide (NBS, Sigma) was recrystallized from

boiling water and dried for two days under reduced pressure over anhydrous calcium chloride. Triphenylphosphine (Ph_3P, Strem) and carboxylic anhydride (Acros) were used as received. Dimethylacetamide (DMAc, Fisher) and dimethyl sulfoxide (DMSO, Acros) were kept over 4 Å molecular sieves and stored under dry nitrogen until use. Acetone (Fisher), anhydrous tetrahydrofuran (THF, Sigma), and methanol (Fisher) were used as received.

Measurements

^{13}C NMR spectra were obtained on Varian INOVA or Varian UNITY 400 MHz spectrometers with a minimum of 5,000 scans in $CDCl_3$. Chemical shifts are reported relative to the solvent peaks. A Thermo Electron Nicolet 8700 FTIR was used to perform infrared spectroscopic analysis of the samples as pressed KBr pellets. *In situ* FTIR spectra were obtained using a Mettler Toledo ReactIR 45M with a SiComp fiber optic ATR probe. Molecular weight determination was achieved by gel permeation chromatography in N-methylpyrrolidone containing 0.05% lithium bromide using a Waters 1515 isocratic HPLC pump, Viscotek 270 dual detector, and Waters 2414 refractive index detector. Universal calibration curves were prepared using polystyrene standards. All elemental analyses were performed by Atlantic Microlab, Inc. Carbon, hydrogen, and nitrogen contents were performed by Atlantic Microlabs using either Perkin Elmer 2400 II or Carlo Erba 1108 elemental analyzers.

Regioselective bromination and acylation of MCC

The method used to synthesize 6-bromo-6-deoxy-2,3-di-*O*-acyl-cellulose was described in detail in Chapter 3. Briefly, MCC (5 g, 30.8 mmol AGU) was stirred in 225 mL DMAc under nitrogen while the mixture was heated to 160 °C for 1 h. During this time, 25 mL DMAc was distilled from the flask. The slurry was then allowed to cool to 90 °C, at which point 45 g LiBr was added. The contents of the flask were allowed to cool further to room temperature while stirring. The MCC dissolved within 2 h to form a transparent solution. All cellulose solutions were kept under dry nitrogen until use within 24 h.

Ph_3P (32.35 g, 4 eq per AGU) was dissolved in 100 mL dry DMAc. A second solution of NBS (21.95 g, 4 eq per AGU) was made in an additional 100 mL dry DMAc. The Ph_3P solution was added dropwise to the MCC solution, followed by the dropwise addition of the NBS solution.

The reaction solution was heated to 70 °C under nitrogen. After 1 h, 10 eq per AGU of a carboxylic anhydride was slowly added to the reaction, and the flask was stirred overnight at 70 °C. The product was isolated by adding the reaction mixture slowly to 4 L of a 50:50 (v/v) mixture of methanol and deionized water, followed by filtration. The precipitate was twice redissolved in acetone followed by precipitation in ethanol, and then it was dried overnight in a vacuum oven at 50 °C.

Synthesis of 6-azido-6-deoxy-2,3-di-*O*-acyl-cellulose

In a 100 mL 3-necked round-bottom flask, 1.00 g of 6-bromo-6-deoxy-2,3-di-*O*-acyl-cellulose was weighed and dissolved in 50 mL anhydrous DMSO under nitrogen. Then, 5 eq per AGU of NaN_3 was added to the flask and dissolved. The solution was heated to 80 °C and stirred for 24 hours under nitrogen. The product was isolated by pouring the reaction solution into 500 mL deionized water and collecting the precipitate by filtration. The product was redissolved in acetone and then reprecipitated in deionized water, followed by filtration. It was dried in a vacuum oven overnight at 50 °C.

Synthesis of 6-amino-6-deoxy-2,3-di-*O*-acyl-cellulose

In a representative example, 0.500 g of 6-azido-6-deoxy-2,3-di-*O*-acyl-cellulose was dissolved in 50 mL THF, followed by the drop-wise addition of 0.250 mL deionized water. The reaction began as 2 eq per AGU of Ph_3P was added to the flask. The reaction was run under ambient conditions, but the flask was stoppered to prevent solvent loss due to evaporation. The reaction remained homogeneous throughout. After allowing the reaction to run overnight, the solution was transferred to 3,500 MWCO dialysis tubing (pre-wet with water) that was then placed in a large beaker containing ethanol. As the dialysis of the reaction solution against ethanol progressed, a precipitate slowly formed within the tubing. After one day of dialysis, the contents inside the tubing were removed, and the precipitate was isolated by filtration. The precipitate was further purified by Soxhlet extraction with ethanol, and then dried in a vacuum oven at 50 °C.

Synthesis of 6-amido-6-deoxy-2,3-di-*O*-acyl-cellulose

In a 100 mL round-bottom flask, 0.150 g 6-azido-6-deoxy-2,3-di-*O*-acyl-cellulose was dissolved in 5 mL anhydrous DMAc under dry nitrogen, and 20 eq per AGU of a carboxylic anhydride was added to the flask. In a separate flask, 0.290 g Ph_3P (2 eq per AGU) was dissolved in 5 mL DMAc, after which the solution was added to the first flask. The reaction solution was stirred for 16 h at room temperature under dry nitrogen. Afterwards, the solution was transferred to 3,500 MWCO dialysis tubing (pre-wet with water) that was then placed in a large beaker containing ethanol. Dialysis was continued for 3 days, after which the contents of the dialysis tubing were transferred to a round-bottom flask and dried on a rotary evaporator. The product was then dissolved in a minimal amount of acetone, and then precipitated in water. The precipitate was filtered out and dried in a vacuum oven at 50 °C.

Results and Discussion

Synthesis of 6-azido-6-deoxy-2,3-di-*O*-acyl-cellulose

It was shown in Chapter 3 that cellulose can be brominated at carbon 6 (*C*-6, see Figure 2.1) with high selectivity, and that esters of this product have good solubility in organic solvents. The 6-bromo-6-deoxy-2,3-di-*O*-acetyl-cellulose was then reacted with NaN_3 in DMSO at 80 °C to produce 6-azido-6-deoxy-2,3-di-*O*-acetyl-cellulose (Scheme 5.1). The azidation reaction progress was monitored by removal of aliquots of the reaction solution at predetermined lengths of time, and the reaction product was isolated for molecular weight and elemental analysis. The results of this experiment are shown in Table 5.1. After 4 h, the DS of the bromide has been reduced to 0.08, and then further decreases to near zero after 48 hours. The DS of the azide levels off around 0.92 after 12 h. The reaction appears to have some effect on the molecular weight of the cellulose derivative, as its degree of polymerization (DP) has been reduced from 66 in the starting material to 50 for the product after 48 h.

Table 5.1 Homogeneous azide displacement on 6-bromo-6-deoxy-2,3-di-*O*-acetyl-cellulose in DMSO at 80 °C

	DS[1]			
Reaction Time (h)	Br	N_3	Total	DP[2]
0	1.04	0	1.04	66
4	0.08	0.85	0.93	58
8	0.05	0.86	0.91	54
12	0.04	0.92	0.96	62
24	0.02	0.91	0.93	47
36	0.01	0.92	0.93	47
48	< 0.01	0.91	0.91	50

[1)] Determined by elemental analysis

[2)] Determined by GPC

The ^{13}C NMR spectra of 6-azido-6-deoxy-2,3-di-*O*-acetyl-cellulose (Figure 5.2) shows the chemical shift for *C*-6 at 50 ppm, moved from 32 ppm in the brominated starting material. No trace of the brominated carbon remains in the spectrum for the product. The NMR spectrum of the product also lends support to the claim that this reaction is entirely regioselective since different substituents at carbon 2 (*C*-2) are known to cause splitting in the signal for the anomeric carbon (*C*-1). No such splitting is evident here. The FTIR spectrum (Figure 5.3) shows a strong azide absorption at 2110 cm^{-1} and the carbonyl absorption from the ester at 1761 cm^{-1}, indicating the successful incorporation of the azide without significant loss of the ester groups.

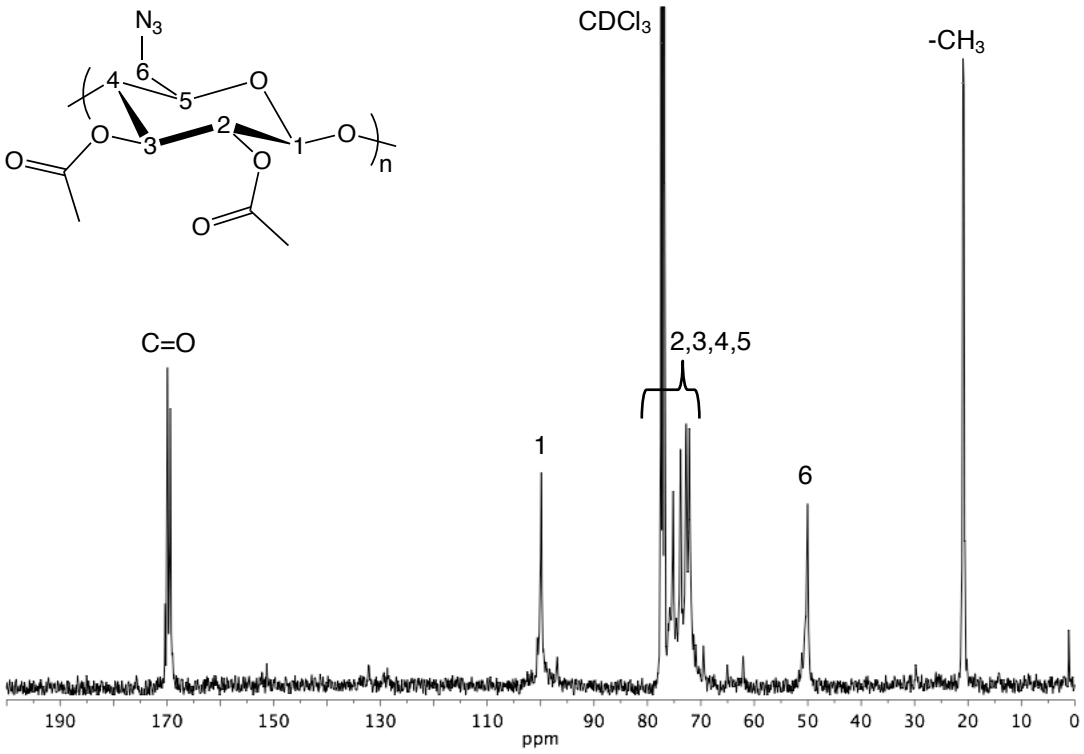


Figure 5.2 ^{13}C NMR spectra 6-azido-6-deoxy-2,3-di-*O*-acetyl-cellulose

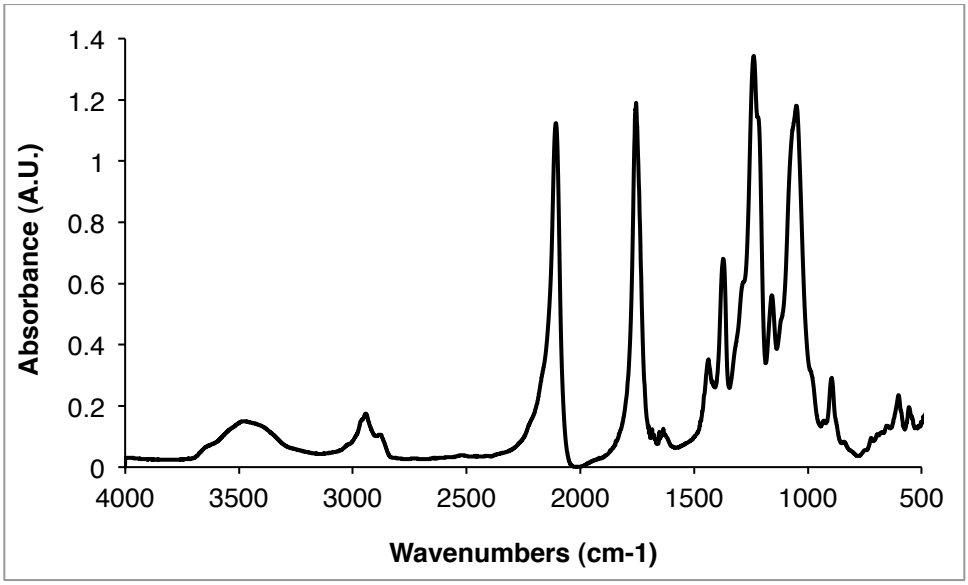


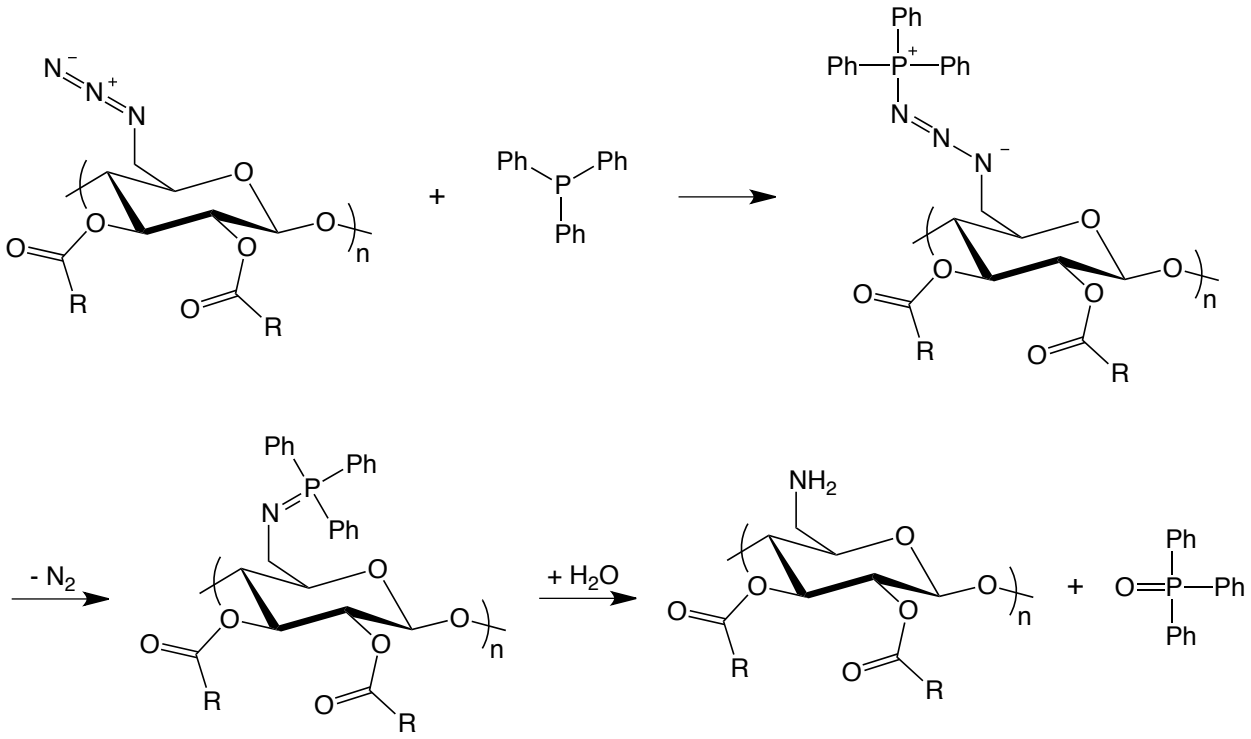
Figure 5.3 FTIR spectrum of 6-azido-6-deoxy-2,3-di-*O*-acetyl-cellulose

In a similar reaction, Liu and Bauman (2002) have previously studied the synthesis of 6-azido-6-deoxy-cellulose from tosylated cellulose. The tosylation of cellulose has been frequently used as a pathway for the regioselective synthesis of cellulose derivatives since substitution occurs preferentially at *C*-6 (Fox, et al. 2011). However, it is known that a tosyl DS of about 1.4 is necessary before the *C*-6 position is completely substituted (Rahn, et al. 1996), and thus tosylation is less regioselective than the bromination pathway used in our experiments. It was found that by reacting tosylated cellulose with 5 equivalents of NaN_3 in DMSO at 50 °C, complete substitution of the tosyl groups at *C*-6 could be achieved in 38 h. Reactions run for 24 h or less were found to contain residual tosyl groups with a DS of at least 0.10 at the *C*-6 position, as determined by quantitative ^{13}C NMR. Additionally, reactions run at a temperature of 100 °C with tosyl cellulose as the substrate resulted in azide groups attached to *C*-2 and *C*-3 as well as *C*-6. Therefore, the higher temperature reactions exhibited reduced regioselectivity. With the brominated cellulose substrate, higher reaction temperatures, and thus shorter reaction times, are possible without any loss of regioselectivity since little or no bromide exists at *C*-2 and *C*-3 to act as a leaving group for azide substitution. Our reaction run at 80 °C resulted in nearly complete substitution (bromide DS of 0.02) after only 24 h. Matsui et al. (2005) previously reported that the reaction of non-esterified 6-bromo-6-deoxy-cellulose with NaN_3 and tetrabutylammonium iodide in DMSO at 70 °C is complete in 48 h, though they did not report any characterization of the product after shorter reaction times. In their case, the reaction did not become homogeneous until after 24 h since 6-bromo-6-deoxy-cellulose is not soluble in DMSO. Therefore, it is likely that their reaction initially proceeded more slowly than when the readily soluble esterified substrate was used as the starting material in our reaction.

Selective azide reduction to produce 6-amino-6-deoxy-2,3-di-*O*-acetyl-cellulose

Our attempts to synthesize an *O*-acetylated amino polysaccharide led us to the use of the Staudinger reaction to selectively and mildly reduce azides to amines. The reaction is illustrated in Scheme 5.2. In it, the 6-azido-6-deoxy-2,3-di-*O*-acyl-cellulose reacts with Ph_3P to form an phosphazide, which in turn loses N_2 to form an iminophosphorane. The iminophosphorane is then hydrolyzed by water to produce 6-amino-6-deoxy-2,3-di-*O*-acyl-cellulose and triphenylphosphine oxide. The reaction was performed by dissolving 6-azido-6-deoxy-2,3-di-*O*-acyl-cellulose in an appropriate solvent and adding 2 equivalents per AGU of Ph_3P. It was run at

room temperature, and the reactants remained in solution when either THF or DMAc was used as the solvent. On the other hand, a precipitate quickly formed after addition of Ph_3P when acetonitrile or ethyl acetate was used as the solvent. The reaction progress in THF was monitored *in situ* with an FTIR probe by observing the disappearance of the azide absorption at 2109 cm^{-1}. Figure 5.4 shows a waterfall plot consisting of a series of FTIR spectra from the reaction obtained over the course of 20 h. In it, the rapid disappearance of the azide peak upon initiation of the reaction (addition of Ph_3P) is readily apparent. For a clearer illustration of this, the absorption intensity at 2109 cm^{-1} versus reaction time has been plotted in Figure 5.5. The reaction proceeds quickly during the first 2 h, and the azide absorption is indistinguishable from the baseline after about 8 h. The selectivity of the reduction is apparent in the waterfall plot with the constant peak height at 1759 cm^{-1}, which represents the carbonyl absorption from the acetate groups. Thus, the secondary hydroxyl groups on the cellulose backbone remain esterified. The peak at 1975 cm^{-1} is due to the presence of THF.



Scheme 5.2 The selective reduction of 6-azido-6-deoxy-2,3-di-*O*-acyl-cellulose to 6-amino-6-deoxy-2,3-di-*O*-acyl-cellulose with Ph_3P

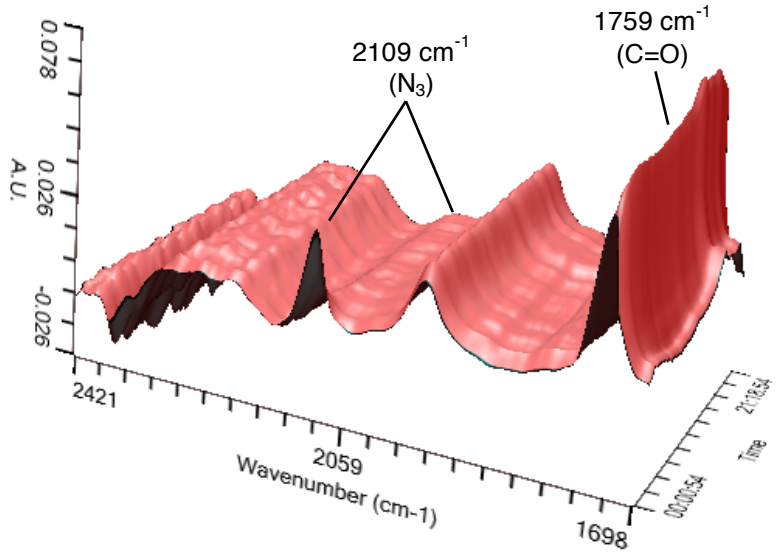


Figure 5.4 A waterfall plot of FTIR spectra vs. reaction time from the Staudinger reduction of 6-azido-6-deoxy-2,3-di-*O*-acetyl-cellulose to 6-amino-6-deoxy-2,3-di-*O*-acetyl-cellulose

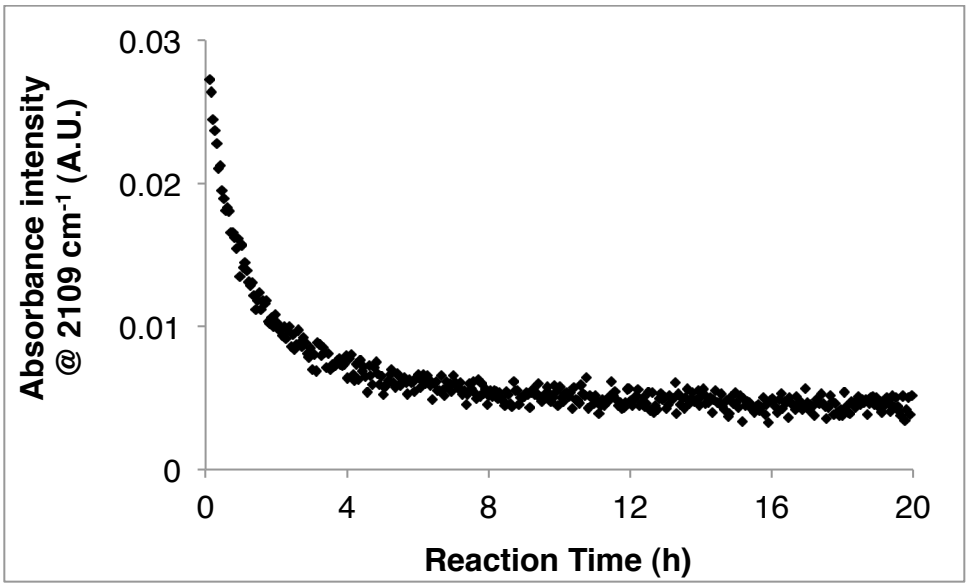


Figure 5.5 A plot of the peak height at 2109 cm^{-1} vs. time, indicating the progress of the azide reduction

Interestingly, once 6-amino-6-deoxy-2,3-di-*O*-acetyl-cellulose was isolated from the reaction solution, it was found to be insoluble in all solvents we tested, including water, 1% aqueous HCl, methanol, DMSO, *N*-methyl-2-pyrrolidinone, acetone, and DMAc with 10% (w/v)

LiCl. The reduction reaction was also performed on propionyl and butyryl esters of the 6-azido-6-deoxy-cellulose to determine if increasing the length of the aliphatic esters by one or two carbons affected the solubility of the 6-amino-6-deoxy-cellulose ester products. However, while the reactions were successful, no difference in the solubilities of the compounds was found.

Due to this poor solubility, solution state NMR of the product had to be obtained prior to isolation from the reaction solvent. Figure 5.6 shows the ^{13}C NMR spectra of the starting material (6-azido-6-deoxy-2,3-di-*O*-acetyl-cellulose) and the reduction product (6-amino-6-deoxy-2,3-di-*O*-acetyl-cellulose) in deuterated THF (d_8-THF). The spectrum of the starting material was obtained prior to the addition of Ph_3P. After the reaction, the chemical shift for *C*-6 has been moved upfield from 51 ppm to 43 ppm. The signals for the carbonyl and methyl carbons in the acetate groups have been retained in the spectrum, further confirming that the ester groups are not reduced. The large peaks between 125 and 140 ppm in the spectrum for the product are due to the presence of Ph_3P and triphenylphosphine oxide (Ph_3PO) in the reaction solution. Further structural information can be obtained from the FTIR spectrum of the isolated product in Figure 5.7. This spectrum has the benefit of a much higher signal to noise ratio for the product than the spectrum obtained *in situ* due to the lack of interference from the presence of solvent. A residual amount of the azide is clearly present at 2110 cm^{-1}, though the peak intensity is greatly reduced from that found in the starting material (Figure 5.3). The residual azide peak was present in samples that had undergone extended reaction times of up to 48 h. The presence of the free amine is evident in the broad N-H stretching band at 3390 cm^{-1} and the N-H bend at 1570 cm^{-1}. The spectrum also shows a weak absorption band at 1669 cm^{-1}, an area that is typically associated with amides (Figure 5.7, spectrum B). It was originally hypothesized that this may be caused by the intermolecular nucleophilic attack of the free amine on the carbonyl carbon of the esters present on the cellulose chain. However, it was later found that the amide band is even more pronounced if the early stages of the reduction reaction are run under anhydrous conditions for 12 hours before the addition of water to hydrolyze the iminophosphorane intermediate (Figure 5.7, spectrum A). This suggests that the iminophosphorane intermediate formed during the reduction reaction may be the more reactive nucleophile in the formation of the amides. Both compounds used to obtain the FTIR spectra in Figure 5.7 were obtained by reducing samples of the same starting material with the same mass of the reducing agent (Ph_3P), for the same duration (24 h), in the same solvent (DMAc). The only

difference was that the reaction for the product in spectrum A was run under anhydrous conditions for the first 12 h prior to the addition of water, while water was added prior to the addition of the Ph_3P in the reaction for the product in spectrum B. Without the presence of water to hydrolyze the iminophosphorane intermediate to produce the free amine, the iminophosphorane was able to persist in the reaction and react with the esters to transfer the acyl group from the oxygen to the nitrogen. In the presence of water, the hydrolysis of the iminophosphorane is presumably faster than the acyl transfer reaction. The reactivity of iminophosphoranes with carbonyl carbons has been well documented in the literature, and in fact is the basis of the Staudinger ligation, a variation on the Staudinger reduction used to connect two different molecules through an amide bond (Saxon and Bertozzi 2000). Other than the intensity of the band at 1670 cm^{-1}, the two FTIR spectra are nearly identical.

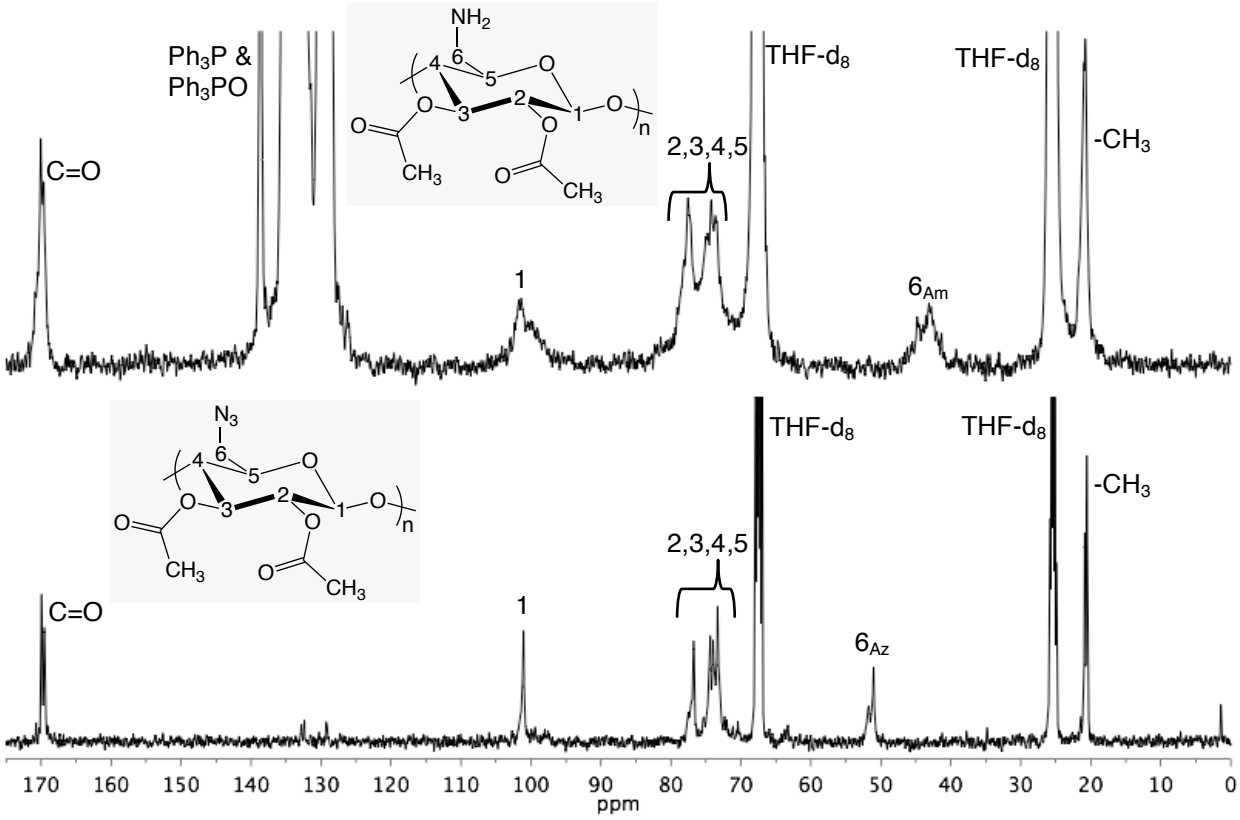


Figure 5.6 ^{13}C NMR spectra of 6-azido-6-deoxy-2,3-di-*O*-acetyl-cellulose (bottom) and 6-amino-6-deoxy-2,3-di-*O*-acetyl-cellulose (top) in d_8-THF. The top spectrum also shows the presence of excess Ph_3P and triphenylphosphine oxide (Ph_3PO) byproduct

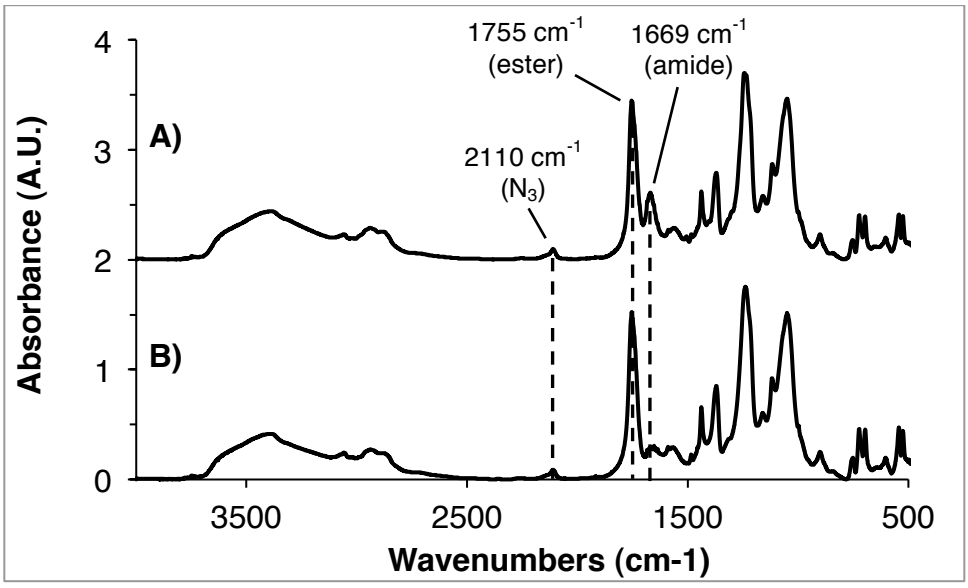
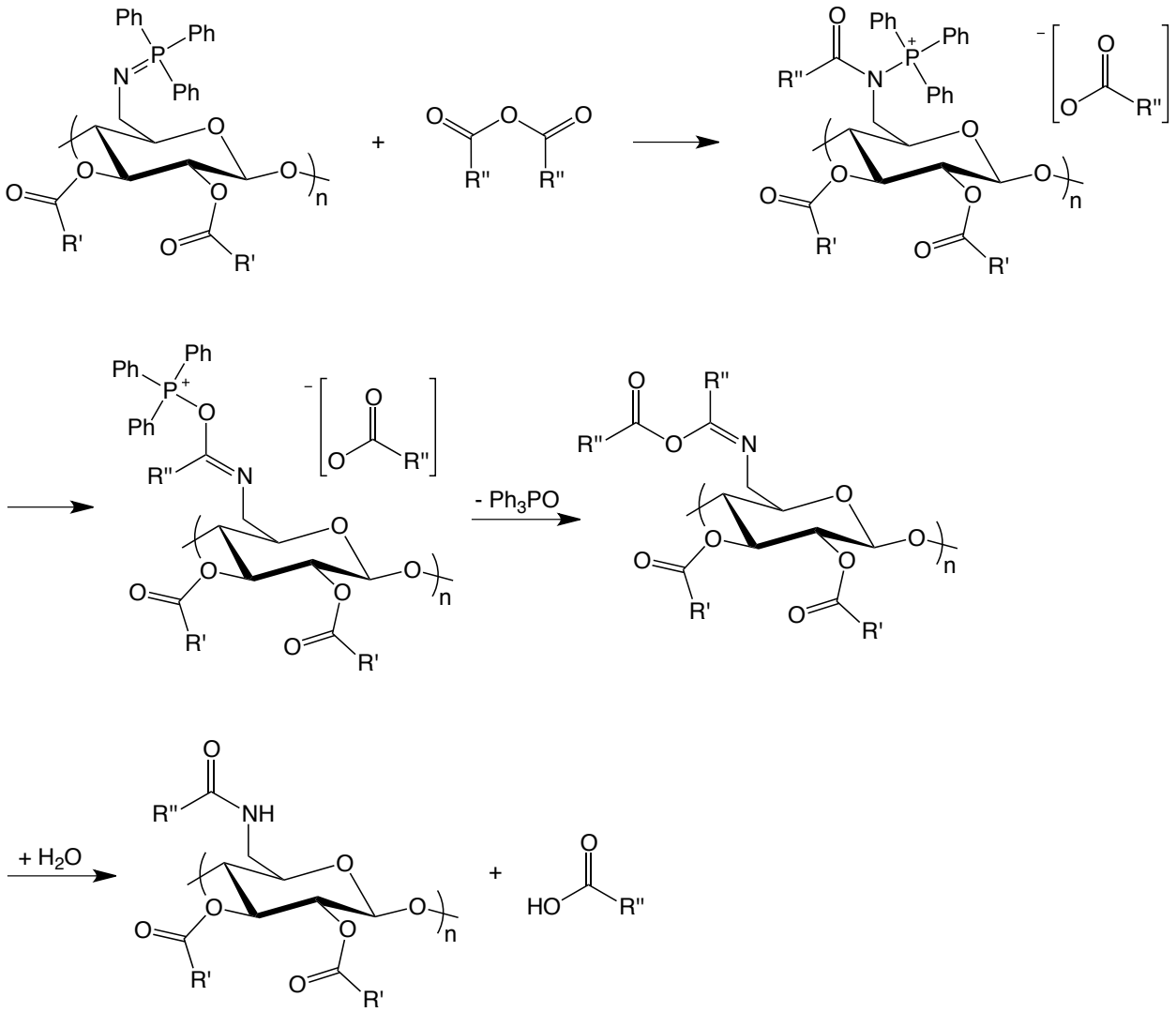


Figure 5.7 FTIR spectra of **A)** 6-amino-6-deoxy-2,3-di-*O*-acetyl-cellulose synthesized under anhydrous conditions during the first 12 h of reaction, followed by the addition of water and **B)** 6-amino-6-deoxy-2,3-di-*O*-acetyl-cellulose synthesized with excess water present throughout the course of the reaction

Synthesis of 6-amido-6-deoxy-2,3-di-*O*-acyl-cellulose

The susceptibility of the iminophosphorane intermediate formed during the synthesis of 6-amino-6-deoxy-2,3-di-*O*-acyl-cellulose to form amide bonds presented an opportunity to create a new regioselectively acylated cellulose derivative. By reducing the 6-azido-6-deoxy-2,3-di-*O*-acyl-cellulose with Ph_3P under anhydrous conditions in the presence of an excess of a carboxylic anhydride, an acyl group different from that attached to the *C*-2 and *C*-3 positions through an ester bond could be attached to the *C*-6 position through an amide bond. Similar conversions of azides to amides have previously been reported (Garcia, et al. 1984; Bosch, et al. 1996). The proposed mechanism, supported by research by Bosch et al. (1996) using ^{31}P NMR, is depicted in Scheme 5.3. Beginning with the iminophosphorane shown in Scheme 5.2, the nitrogen atom attacks an electrophilic carbonyl carbon in the carboxylic anhydride. The positively charged phosphonium species then migrates to the carbonyl oxygen of the newly formed amide, now creating a new imine. Triphenylphosphine oxide is then eliminated as the acyl anion attacks the imino carbon. The resulting species is then readily hydrolyzed to an amide upon exposure to

moisture. In this manner, 6-acetamido-6-deoxy-2,3-di-*O*-acetyl-cellulose, 6-acetamido-6-deoxy-2,3-di-*O*-propionyl-cellulose, and 6-propionamido-6-deoxy-2,3-di-*O*-acetyl-cellulose were synthesized. The reactions were run in anhydrous DMAc because a precipitate formed upon the addition of the Ph_3P to the reaction solution of the starting cellulose azide and carboxylic anhydride if THF was the solvent. The products were soluble in acetone and chloroform. The ^{13}C NMR spectra of these compounds are shown in Figures 5.8, 5.9, and 5.10. In each case, the chemical shift for *C*-6 with a pendant amide group is found at 46 ppm, whereas the aminated *C*-6 in Figure 5.6 occurs at 43 ppm. The carbonyl and aliphatic carbons of the amide group are shifted downfield from their ester counterparts. The chemical shifts for *C*-6 and the carbons in the acetate and propionate groups are listed in Tables 5.2 and 5.3. Additional information about the ^{13}C NMR chemical shifts in cellulose esters can be found in the monograph by Heinze et al. (2006b). Each ^{13}C NMR spectrum of the 6-amido-6-deoxy-cellulose esters also still have peaks between 128 ppm and 135 ppm that are likely due to aryl phosphines still in the samples. These peaks are persistent even after thorough washing of the samples and extended dialysis with ethanol, which may mean that the peaks are from carbon nuclei still covalently bonded to the cellulose derivatives. It may be the case that some of the iminophosphorane intermediates in the reactions still persist on the cellulose. This matter needs to be investigated further to positively identify the source of these peaks. An additional unexplained peak present in each of the ^{13}C NMR spectra occurs at 100.5 ppm, near the peak assigned to *C*-1. The sharpness of this peak would suggest that it is an impurity in the sample, but it is also persistent through multiple attempts to purify the sample. The FTIR spectrum of 6-acetamido-6-deoxy-2,3-di-*O*-propionyl-cellulose is shown in Figure 5.11. In it, a strong amide absorption is clearly visible at 1700 cm^{-1}.



Scheme 5.3 Proposed mechanism for the *N*-acylation of 6-deoxy-6-iminophophorane-cellulose esters

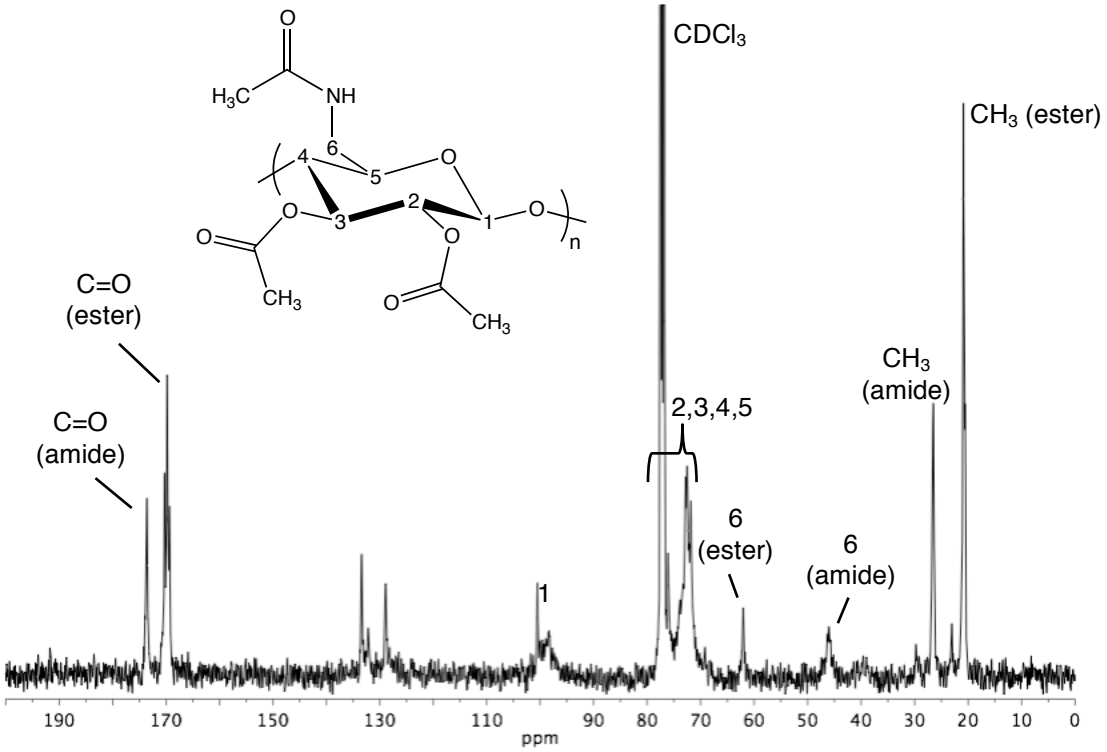


Figure 5.8 ^{13}C NMR spectrum of 6-acetamido-6-deoxy-2,3-di-*O*-acetyl-cellulose

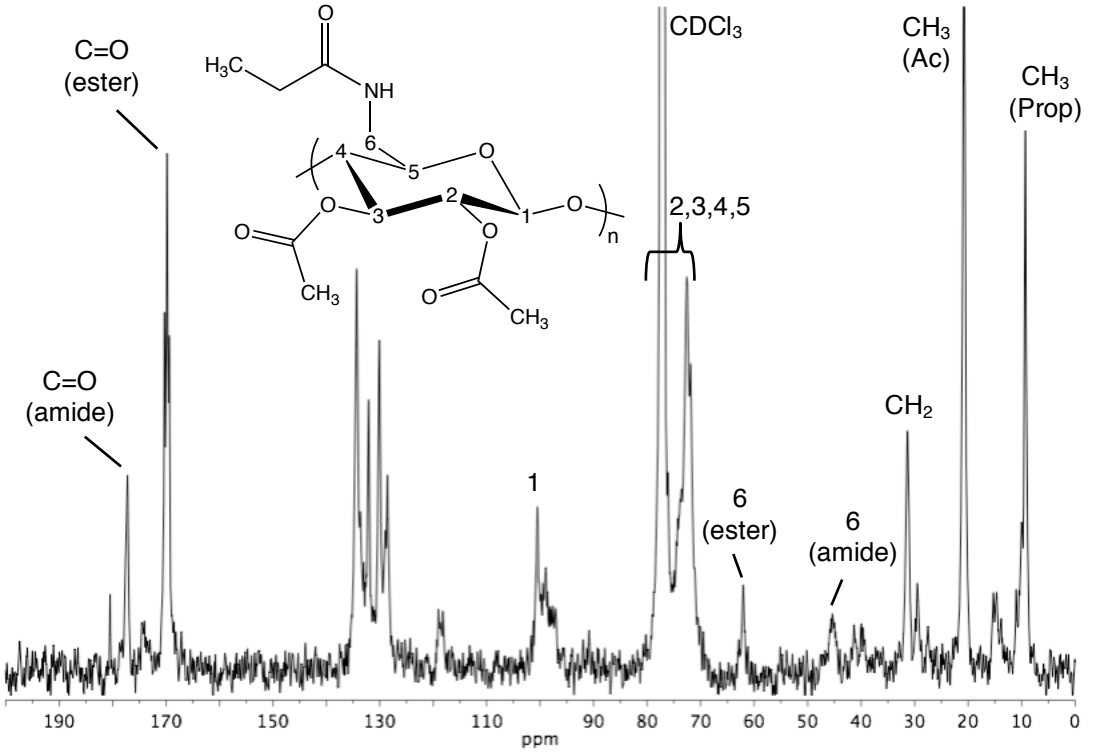


Figure 5.9 ^{13}C NMR spectrum of 6-propionamido-6-deoxy-2,3-di-*O*-acetyl-cellulose

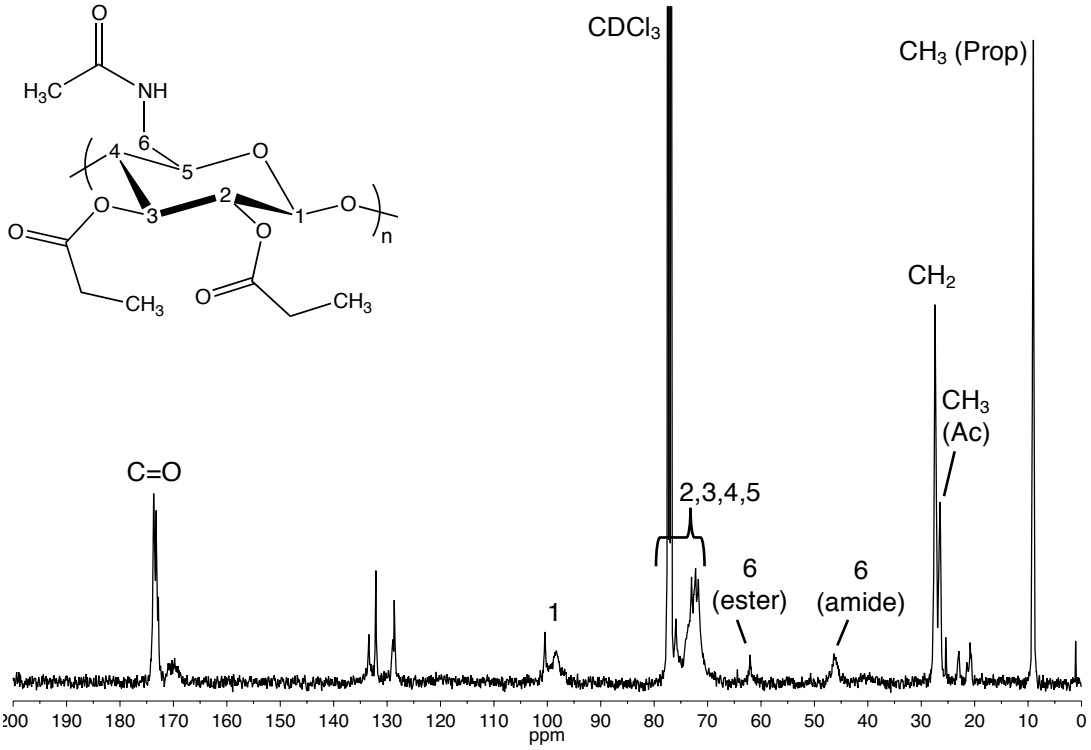


Figure 5.10 ^{13}C NMR spectrum of 6-acetamido-6-deoxy-2,3-di-*O*-propionyl-cellulose

Table 5.2 ^{13}C NMR chemical shift assignments for *C*-6 with different substituents determined in $CDCl_3$

C-6 substituent	δ (ppm)
ester	62.0
amine	43.0*
amide	46.0

* determined with d_8-THF as the NMR solvent

Table 5.3 ^{13}C NMR chemical shift assignments for acetyl and propionyl groups in 6-amido-6-cellulose esters determined in $CDCl_3$

Carbon	δ (ppm)	Carbon	δ (ppm)
Acetyl		Propionyl	
CH_3 (ester)	20.8	CH_2 (ester)	27.4
CH_3 (amide)	26.5	CH_2 (amide)	31.3
C=O (ester)	169.8	CH_3 (ester)	9.0
C=O (amide)	173.6	CH_3 (amide)	9.3
		C=O (ester)	173.5
		C=O (amide)	177.2

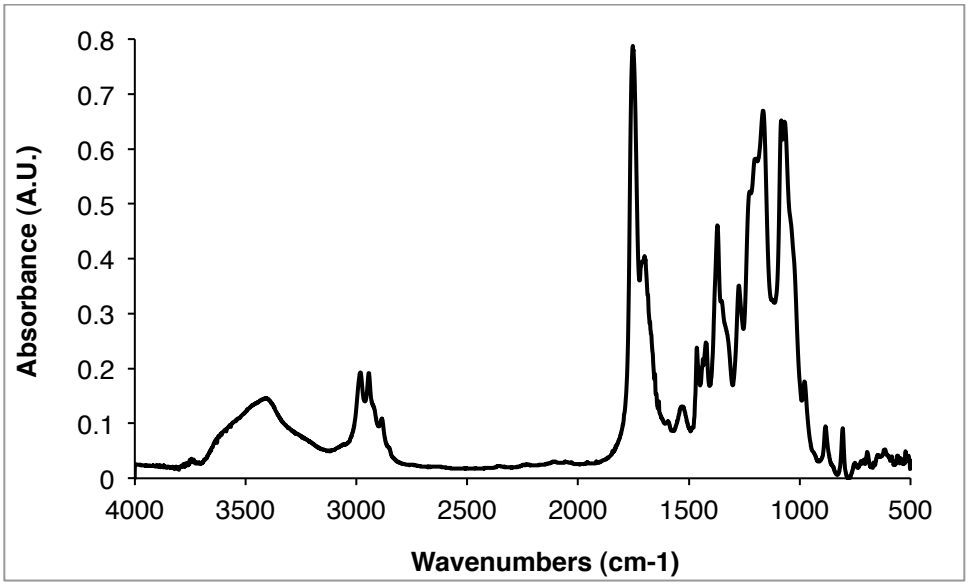


Figure 5.11 FTIR spectrum of 6-acetamido-6-deoxy-2,3-di-*O*-propionyl-cellulose

Conclusions

A new method for synthesizing a selectively *O*-acylated amino polysaccharide has been created. First, 6-azido-6-deoxy-2,3-di-*O*-acyl-cellulose was synthesized from 6-bromo-6-deoxy-2,3-di-*O*-acyl-cellulose by reaction with NaN_3 in DMSO. This step represents an improvement over previously reported azide displacement reactions on cellulose due to its high regioselectivity

and shorter reaction times. The azide was then selectively and mildly reduced using Ph_3P at room temperature. This reduction step resulted in little or no loss of ester groups attached at *C*-2 and *C*-3 on cellulose. The structure of the product was confirmed by ^{13}C NMR and FTIR. The 6-amino-6-deoxy-2,3-di-*O*-acyl-cellulose was not soluble in any of the solvents in which it was tested. To create a soluble derivative, the 6-azido-6-deoxy-2,3-di-*O*-acyl-cellulose was reduced with Ph_3P under anhydrous conditions in the presence of carboxylic anhydrides, which resulted in the formation of amides at *C*-6. By using a carboxylic anhydride that reacts to form a different *N*-acyl group than what is already attached to the *C*-2 and *C*-3 positions, a new regioselectively acylated cellulose derivative was synthesized.

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CHAPTER 6: SYNTHESIS OF REGIOSELECTIVELY IODINATED CELLULOSE DERVATIVES

Abstract

Regioselectively iodinated cellulose derivatives were synthesized by reacting 6-bromo-6-deoxy-cellulose esters with sodium iodide under Finkelstein reaction conditions. Similar reactions have been performed previously on tosylated cellulose, but the method described here results in higher selectivity of the iodide displacement for *C*-6. The 6-deoxy-6-iodo-cellulose esters were synthesized because iodide is generally a better leaving group than bromide in nucleophilic substitution reactions, and therefore the iodinated cellulose might be better suited to reactions with weak nucleophiles. Triethylamine and triethylphosphine were used as nucleophiles in reactions intended to test this hypothesis on cellulose derivatives, though more data is needed to conclude whether or not the iodinated cellulose is the better substrate for substitution reactions.

Introduction

In the past couple of decades, tosylated and halogenated cellulose derivatives have proven useful in the synthesis of new functional materials (Aoki, et al. 1998; Koschella, et al. 2006; Petzold-Welcke, et al. 2009; Fox, et al. 2011). This is due to the ability of tosylate and halide anions to act as good leaving groups, allowing a variety of chemical functional groups to be directly attached to the cellulose backbone via nucleophilic substitution. Additionally, tosylates and halides can be attached preferentially to carbon 6 (*C*-6, see Figure 2.1) on each anhydroglucose unit (AGU) in cellulose, providing a mechanism by which the new functional groups can be regioselectively introduced to cellulose. These regioselective derivatization reactions are of particular interest to cellulose chemists because it has been shown that the distribution of substituents along a cellulose backbone can affect the physical properties of a derivative.

While it has been shown that the bromination of cellulose can be made to occur with much higher regioselectivity for *C*-6 than tosylation (Fox, et al. 2011), tosyl cellulose has been

used more frequently than brominated cellulose for the synthesis of new functional derivatives of cellulose. This is likely due in part to the fact that tosyl cellulose has higher solubility in a range of organic solvents than brominated cellulose. This problem was recently overcome by the *in situ* acylation of 6-bromo-6-deoxy-cellulose, resulting in derivatives with very high structural regularity and good organic solubility (Fox and Edgar 2011). The utility of these brominated cellulose esters was subsequently demonstrated by using them in the synthesis of 6-azido- and 6-cyano-6-deoxy-cellulose esters (see Chapters 4 and 5).

One advantage that tosyl cellulose may still hold over the brominated cellulose, however, is that the tosyl anion is generally a superior leaving group compared to a bromide anion in nucleophilic substitution reactions (Carey and Sundberg 2007). Thus, tosyl cellulose may still be the superior substrate in reactions where weak nucleophiles are to be used, though this hypothesis has not yet been systematically investigated for polysaccharide substrates. One potential solution for improving the reactivity of the halogenated cellulose would be to substitute the bromide in 6-bromo-6-deoxy-cellulose with iodide, which has properties as a leaving group more comparable to those of the tosylate anion. Thus, a new cellulose derivative synthesized with high regioselectivity and potentially good reactivity with weak nucleophiles could be produced.

Precedent for the iodide substitution reaction exists in previous research performed with tosylated cellulose. The iodide displacement of tosyl groups using ketone solvents (Finkelstein reaction conditions) has been used on many occasions as an analytical tool to determine the position of substitution of the tosylates on cellulose (Cramer and Purves 1939; Malm, et al. 1948; Liu, et al. 1993; Rahn, et al. 1996). It was thought that tosyl groups attached to the *C*-6 position could be quantitatively substituted with iodide by an S_N2 displacement reaction, while tosyl groups attached to carbons 2 and 3 (*C*-2 and *C*-3) would be left intact due to the high energy barriers to S_N2 reactions at those positions. However, an investigation by Rahn et al. (1996) showed residual tosyl groups remain at *C*-6 and a small amount of substitution of the iodide does occur at *C*-2 (see Chapter 4 and Figure 4.3 for additional discussion). Liu and Baumann (2002) later discovered that by lowering the reaction temperature from 120 °C to 60 °C and prolonging the reaction time from 2 h to 42 h, quantitative and selective iodide displacement of tosyl groups at *C*-6 could in fact be achieved.

To date, however, there have been no reports on the synthesis of 6-deoxy-6-iodo-cellulose from 6-bromo-6-deoxy-cellulose, and no iodinated cellulose has been used as a substrate in the synthesis of functional cellulose derivatives. This chapter will discuss the first synthesis of 6-deoxy-6-iodo-2,3-di-*O*-acyl-cellulose, and then it will briefly present data obtained from the reactions of 6-deoxy-6-iodo-2,3-di-*O*-acetyl-cellulose with triethylamine and triethylphosphine.

Experimental

Materials

Microcrystalline cellulose (MCC, Avicel® PH-101, Fluka) was dried under reduced pressure at 50 °C overnight prior to use. Lithium bromide (LiBr, Fisher) and sodium iodide (NaI, Fisher) were dried under reduced pressure at 125 °C. N-Bromosuccinimide (NBS, Sigma) was recrystallized from boiling water and dried for two days under reduced pressure over anhydrous calcium chloride. Triphenylphosphine (Ph_3P, Strem), triethylphosphine (Aldrich), all carboxylic acid anhydrides (Acros), and phenyl isocyanate (Acros) were used as received. Acetylacetone (Fisher) and triethylamine (Acros) were dried over 4 Å molecular sieves and redistilled prior to use. Dimethylacetamide (DMAc, Fisher) and dimethylformamide (DMF, Fisher) were kept over 4 Å molecular sieves and stored under dry nitrogen until use. Ethanol, acetone, and methanol (all from Fisher) were used as received.

Measurements

NMR spectra were obtained on Varian INOVA or UNITY 400 MHz spectrometers in $CDCl_3$ or d6-DMSO. ^{13}C NMR spectra were obtained with a minimum of 5000 scans. Chemical shifts are reported relative to the solvent peaks. Molecular weight determination was achieved by gel permeation chromatography in N-methylpyrrolidone containing 0.05% lithium bromide using a Waters 1515 isocratic HPLC pump, Viscotek 270 dual detector, and Waters 2414 refractive index detector. Universal calibration curves were prepared using polystyrene standards. All elemental analyses were performed by Atlantic Microlab, Inc. Carbon, hydrogen, and nitrogen contents were determined using either Perkin Elmer 2400 II or Carlo Erba 1108 elemental

analyzers. Bromine and iodine contents were determined by flask combustion followed by ion chromatography.

Regioselective bromination and acylation of MCC

The method used to synthesize 6-bromo-6-deoxy-2,3-di-*O*-acyl-cellulose was described in detail in Chapter 3. Briefly, MCC (5 g, 30.8 mmol AGU) was stirred in 225 mL DMAc under nitrogen while the mixture was heated to 160 °C for 1 h. During this time, 25 mL DMAc was distilled from the flask. The slurry was then allowed to cool to 90 °C, at which point 45 g LiBr was added. The contents of the flask were allowed to cool further to room temperature while stirring. The MCC dissolved within 2 h to form a transparent solution. All cellulose solutions were kept under dry nitrogen until use within 24 h.

Ph_3P (32.35 g, 4 eq per AGU) was dissolved in 100 mL dry DMAc. A second solution of NBS (21.95 g, 4 eq per AGU) was made in an additional 100 mL dry DMAc. The Ph_3P solution was added dropwise to the MCC solution, followed by the dropwise addition of the NBS solution. The reaction solution was heated to 70 °C under nitrogen. After 1 h, 10 eq per AGU of a carboxylic anhydride was slowly added to the reaction, and the flask was stirred overnight at 70 °C. The product was isolated by adding the reaction mixture slowly to 4 L of a 50:50 (v/v) mixture of methanol and deionized water followed by filtration. The precipitate was twice redissolved in acetone followed by precipitation in ethanol, and then it was dried overnight in a vacuum oven at 50 °C.

Synthesis of 6-deoxy-6-iodo-2,3-di-*O*-acyl-cellulose

In a representative example, 2.5 g 6-bromo-6-deoxy-2,3-di-*O*-acetyl-cellulose and 4.85 g NaI (4 eq. per AGU) were dissolved in 100 mL acetylacetone under nitrogen. The reaction was stirred and heated to 120 °C in an oil bath for 2 h. The solution became cloudy over the course of the reaction. Afterwards, the contents of the reaction flask were allowed to cool and then added to 900 mL 50/50 water/methanol. The product precipitated from solution and was filtered out. It was redissolved in acetone and then reprecipitated in water. The filtered product was dried in the vacuum oven at 50 °C.

Reactions of 6-deoxy-6-halo-cellulose acetates with triethylamine and triethylphosphine

In a 100 mL round-bottom flask, 500 mg of either 6-bromo-6-deoxy-2,3-di-*O*-acetyl-cellulose or 6-deoxy-6-iodo-2,3-di-*O*-acetyl-cellulose was dissolved in 50 mL dried DMF under dry nitrogen. Then 5 eq of triethylamine or triethylphosphine were added to the solution. The reaction was heated in an oil bath to 80 °C for 24 hours. The triethylamine substituted products were isolated by dialysis against water through a 3500 MWCO cellulose membrane. The triethylphosphine substituted products were isolated by dialysis through a 3500 MWCO cellulose membrane first against methanol, and then against water. After dialysis, the samples either dissolved or suspended in water were transferred to a round bottom flask, and the water was then removed on a rotary evaporator. The product was scraped from the sides of the flask and then dried in a vacuum oven at 50 °C.

Results and Discussion

Synthesis of 6-deoxy 6-iodo-cellulose esters

The first attempts in this research project to synthesize an iodinated cellulose derivative substituted with high selectivity at *C*-6 were described in Chapter 3. In those reactions, tosylated cellulose was dissolved in acetylacetone and reacted with NaI at 120 °C to produce an iodinated derivative previously described by Rahn et al. (1996). Analysis of the product by ^{13}C NMR showed that most of the *C*-6 positions in the cellulose were indeed substituted with an iodide (see Figure 4.3). However, iodide substitution also clearly occurred at the *C*-2 position, as indicated in the splitting of the *C*-1 peak. Additionally, significant tosyl substitution was still present at the *C*-2 and *C*-3 positions. This is largely unavoidable for this reaction since a minimum tosyl DS of about 1.4 is necessary for the starting material to be soluble in ketone solvents (Rahn, et al. 1996).

After it was discovered that acylated 6-bromo-6-deoxy-cellulose derivatives displayed good solubility in ketone solvents, it was proposed that they might also be good substrates for the synthesis of regioselectively iodinated cellulose via a similar Finkelstein reaction. It was found that the same reaction conditions did indeed result in high conversion of the bromide to the iodide with good yields of the product. Reactions were run using 6-bromo-6-deoxy-cellulose acetate, propionate, and butyrate esters as substrates. The halogen DS and degrees of

polymerization (DP) for the starting materials and the products of the reactions are shown in Table 6.1. A high iodide DS of 0.95 was obtained from the butyrated starting material that had an initial bromide DS of 1.10. In the other two samples, iodide DS values of 0.79 and 0.80 were obtained from starting materials with initial bromide DS values of 0.95 and 0.94, respectively. The products obtained in each case contained a small residual amount of bromide, with DS_{Br} values between 0.09 and 0.14. A comparison of the DP's of the starting materials and the products indicates that little or no degradation of the cellulose backbone occurs over the course of the reaction.

Table 6.1 Yield, DS, molecular weight results from the synthesis of 6-deoxy-6-iodo-2,3-di-*O*-acyl-cellulose from 6-bromo-6-deoxy-2,3-di-*O*-acyl-cellulose

	Starting Material		Product			
Acyl group	Bromide DS[1]	DP[2]	Iodide DS[1]	Bromide DS[1]	DP[2]	Yield
Acetyl	0.95	85	0.79	0.14	82	92%
Priopionyl	0.94	80	0.80	0.09	82	85%
Butyryl	1.10	84	0.95	0.13	79	89%

[1)] Determined by elemental analysis

[2)] Determined by GPC. The measured DP of the starting cellulose was 80.

The ^{13}C and ^{1}H NMR spectra for 6-deoxy-6-iodo-2,3-di-*O*-acetyl-cellulose are shown in Figures 6.1 and 6.2. All of the proton and carbon NMR spectra for the 6-deoxy-6-iodo-cellulose acetate, propionate, and butyrate esters were nearly identical to those of the corresponding 6-bromo-6-deoxy-cellulose esters except for the peaks assigned to the *C*-6 position. In the ^{13}C NMR spectra, the signal for *C*-6 has been shifted upfield from 32 ppm for the starting material (see Figures 3.4 through 3.6) to 5 ppm for the product. In the ^{1}H NMR spectra, the two peaks found at 3.51 and 3.81 ppm in the starting material (see Figure 6.2) have been shifted upfield to 3.21 and 3.54 ppm in the product, indicating that those peaks can be assigned to the two diastereotopic protons attached to *C*-6.

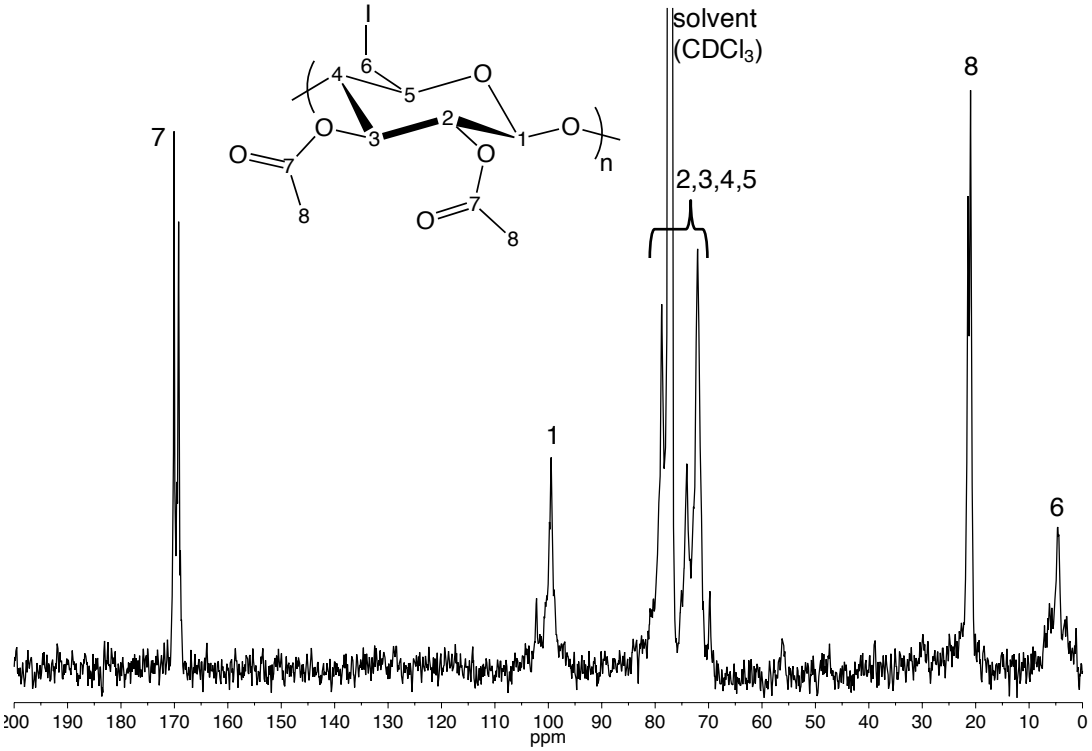

Figure 6.1 ^{13}C NMR of 6-deoxy-6-iodo-2,3-di-*O*-acetyl-cellulose

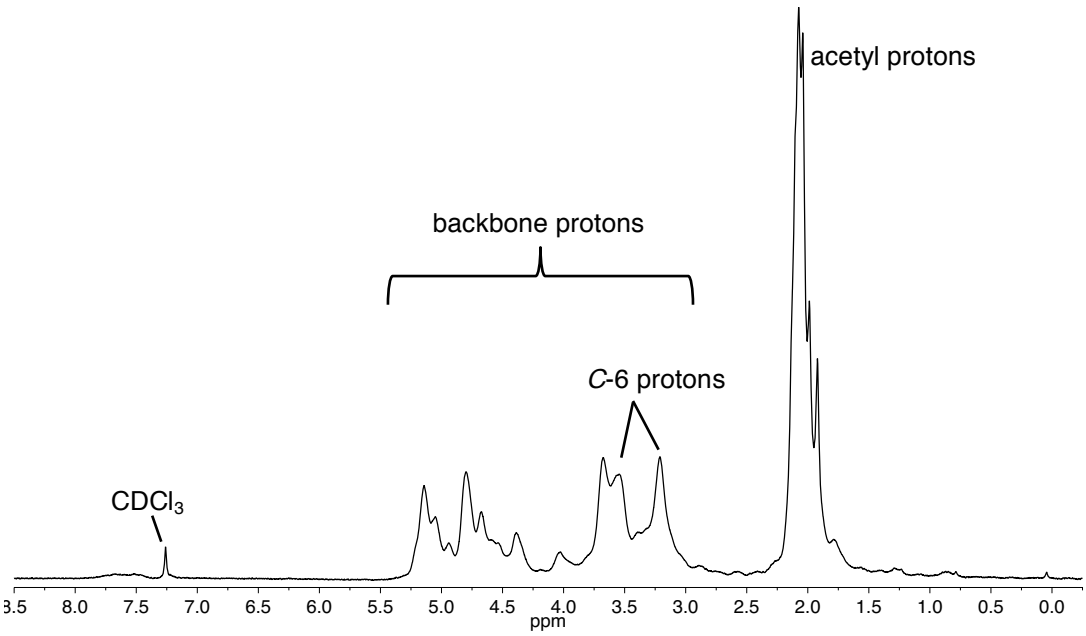

Figure 6.2 ^{1}H NMR of 6-deoxy-6-iodo-2,3-di-*O*-acetyl-cellulose

Displacement reactions with 6-deoxy-6-iodo-2,3-di-*O*-acetyl-cellulose substrates

Attempts were made to compare the reactivity of the 6-bromo-6-deoxy-2,3-di-*O*-acetyl-cellulose to the 6-deoxy-6-iodo-2,3-di-*O*-acetyl-cellulose in substitution reactions with two different nucleophiles, triethylamine and triethylphosphine. A similar reaction between tosyl cellulose and triethylamine in DMF was previously investigated by Koschella and Heinze (2001). By including water in the reaction to partially hydrolyze unreacted tosyl residues still attached to the cellulose, the authors found that they could produce water soluble derivatives with a low DS of the quaternary amine. Reactions run under anhydrous conditions resulted in products that were soluble in DMSO but not in water. In either case, high conversion of the tosyl groups to the quaternary amine was not achieved at reactions lasting 24 h with temperatures up to 100 °C.

The reactions the triethylamine performed here on 6-bromo-6-deoxy-2,3-di-*O*-acetyl-cellulose and 6-deoxy-6-iodo-2,3-di-*O*-acetyl-cellulose in DMF at 80 °C for 24 h resulted in products that were soluble in DMSO but not water or acetone. In the ^{13}C NMR spectrum for the product of the brominated substrate is shown in Figure 6.3, and the corresponding spectrum from the iodinated substrate is shown in Figure 6.4. The halogenated *C*-6 peaks are still present in each spectrum. However, peaks for the methyl (CH_3) and methylene (N-CH_2) carbons of the ethyl groups attached to the quaternized amine are also visible at 7.2 and 53.5 ppm, indicating that some substitution did occur. Additionally, a small peak for the triethylamine substituted *C*-6 is visible at 56.8 ppm in the spectrum in Figure 6.4 (it is likely below the baseline in Figure 6.3). In their paper, Koschella and Heinze (2001) present different assignment for the triethylamine substituted *C*-6 the N-CH_2 peaks as 53.4 ppm and 45.6 ppm, respectively. Based on the spectra obtained from the research presented here, they likely misidentified the N-CH_2 peak as the *C*-6 peak. Their peak at 45.6 ppm may be due to an impurity in their sample since no peak at 45.6 is visible here in Figures 6.3 and 6.4. Quantitative ^{13}C NMR spectra for these derivatives have not been obtained, and overlapping peaks in their ^{1}H NMR spectra complicate its analysis. Therefore, it is impossible to say at this point if significantly more triethylamine substitution occurred on either the 6-bromo-6-deoxy-2,3-di-*O*-acetyl-cellulose or the 6-deoxy-6-iodo-2,3-di-*O*-acetyl-cellulose. Further work is needed to clarify this point.

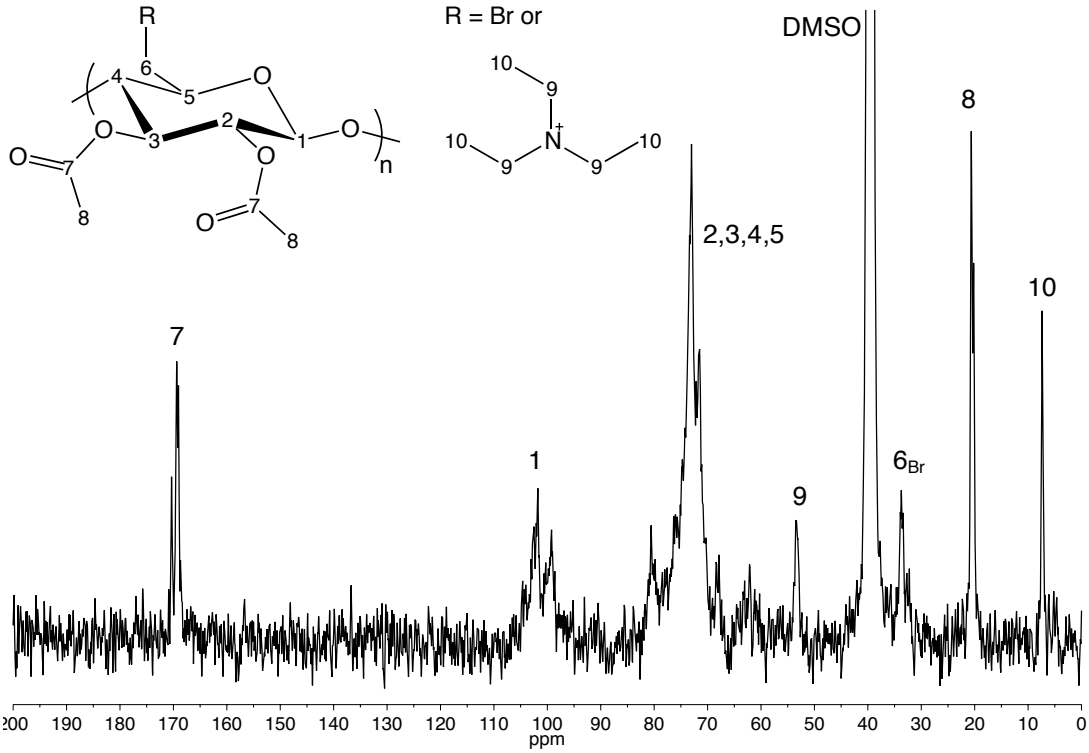

Figure 6.3 ^{13}C NMR spectrum of triethylamine substituted 6-bromo-6-deoxy-2,3-di-*O*-acetyl-cellulose

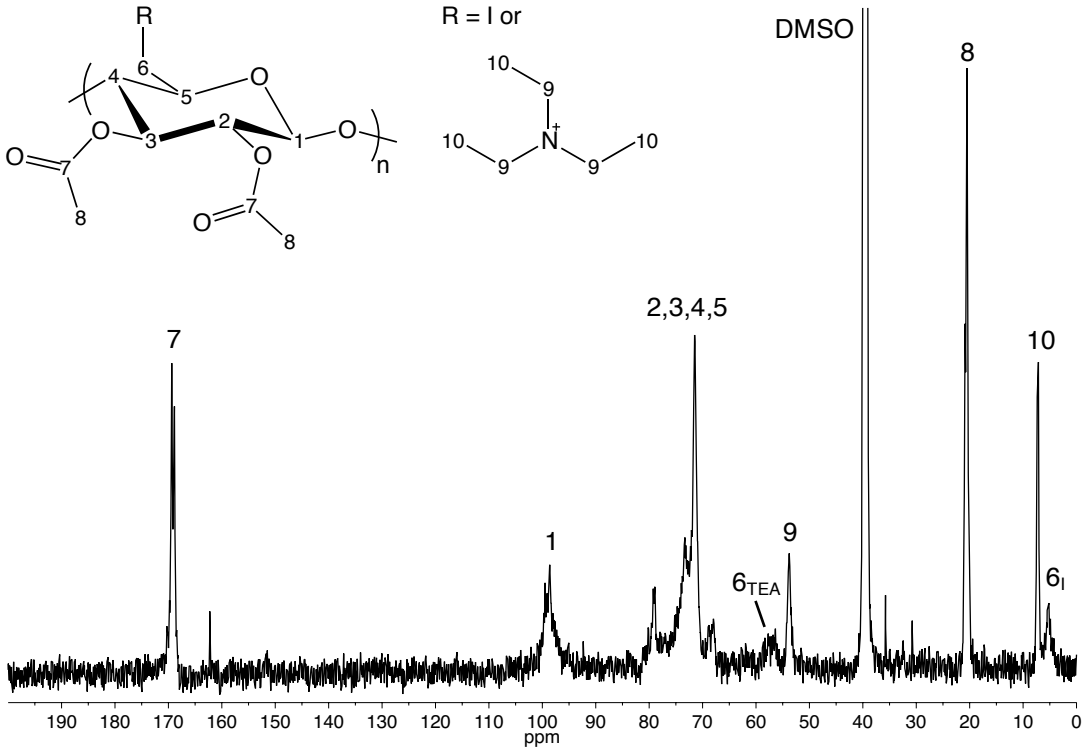

Figure 6.4 ^{13}C NMR spectrum of triethylamine substituted 6-iodo-6-deoxy-2,3-di-*O*-acetyl-cellulose

While there have been many reports on the reactions of nucleophilic amines with tosylated or halogenated cellulose, a search of the literature reveals no reports on similar reactions with phosphine nucleophiles. In this research, triethylphosphine was reacted with 6-deoxy-6-iodo-2,3-di-*O*-acetyl-cellulose under conditions identical to the triethylamine reactions discussed above (80 °C for 24 h in DMF). The product was soluble in water and DMSO. The ^{13}C NMR spectrum of the product indicates that the phosphine was incorporated into the cellulose derivative (Figure 6.5). The peaks at 5.3 ppm and 11.4 ppm are due to the methyl and methylene carbons, respectively, of the ethyl groups bonded to the phosphorus. The phosphine substituted *C*-6 peak is predicted by ACD Labs NMR prediction software to occur at 19.1 ppm, and therefore it may be obscured by the acetyl methyl peak present around 20 ppm. An iodinated *C*-6 peak, if still present, may be obscured by the methyl carbon peak at 5.3 ppm. The sharp peak at 24.7 ppm is likely due to the methylene carbons in triethylphosphine oxide, which is readily formed from excess triethylphosphine upon reaction work-up. The ^{31}P NMR of the product gives additional evidence that the reaction was successful (Figure 6.6). Two peaks show up in the

spectrum at 38.8 ppm and 50.7 ppm. The peak at 50.7 ppm can be assigned to triethylphosphine oxide, and a peak for any residual triethylphosphine would be expected around -20 ppm. Thus, the peak at 38.8 ppm is likely due to the phosphorus atom bonded to *C*-6 in the phosphine substituted cellulose derivative. This conclusion is further supported by the ACD Labs NMR prediction software, which predicts the peak to be around 38 ppm.

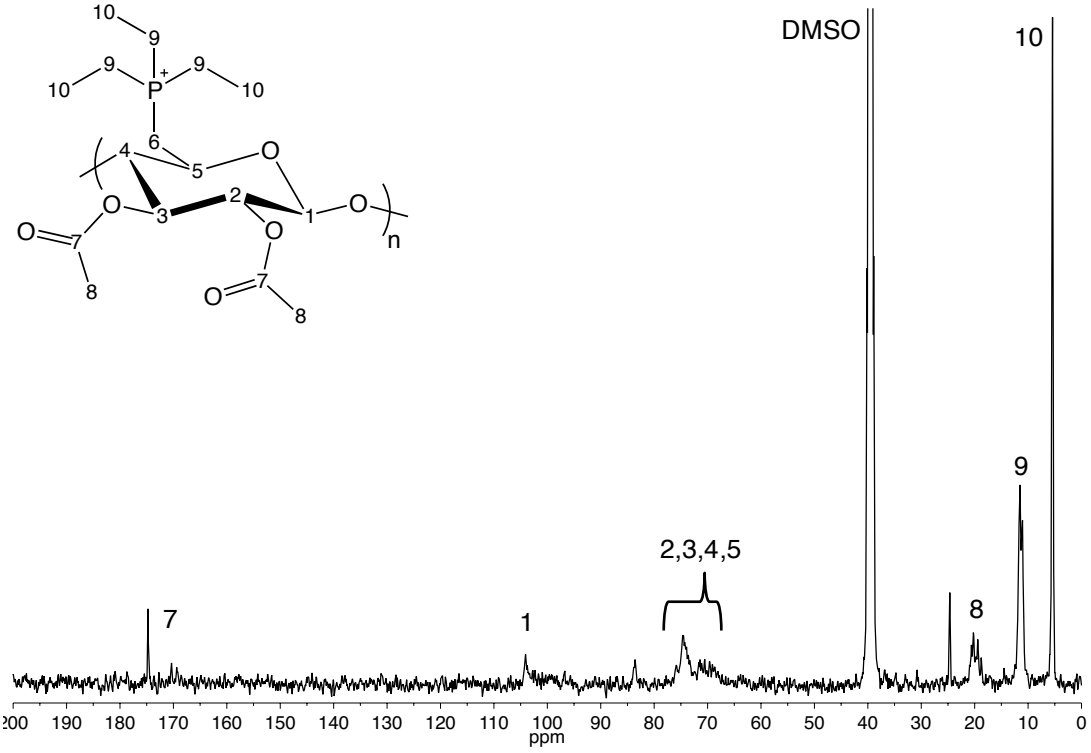

Figure 6.5 ^{13}C NMR spectrum of the product from the reaction of triethylphosphine with 6-deoxy-6-iodo-2,3-di-*O*-acetyl-cellulose

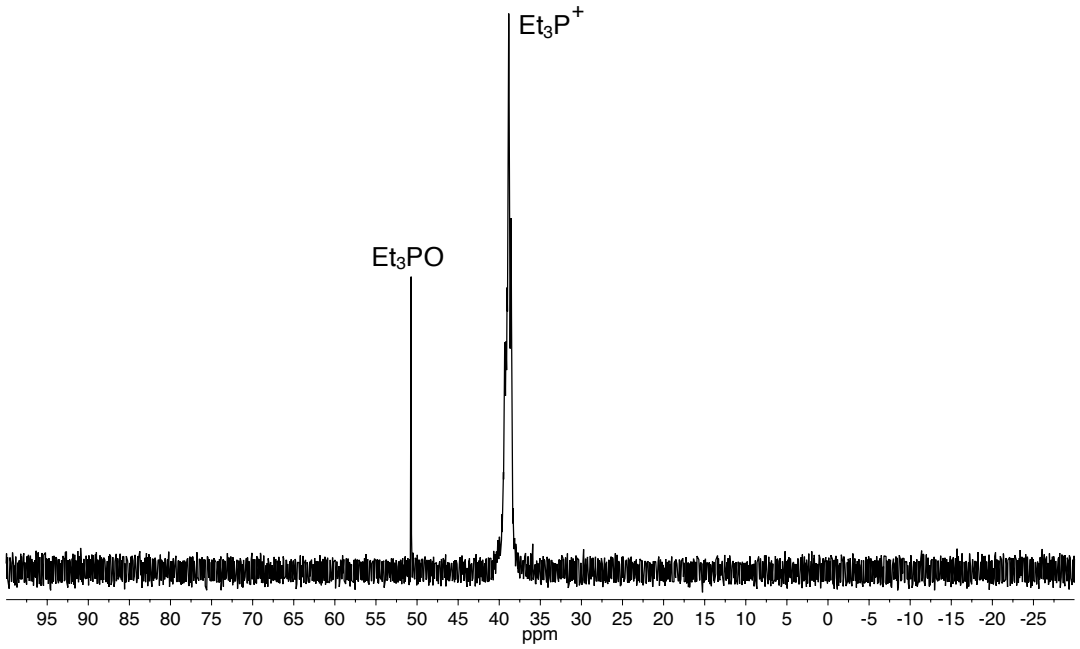

Figure 6.6 ^{31}P NMR spectrum of the product from the reaction of triethylphosphine with 6-deoxy-6-iodo-2,3-di-*O*-acetyl-cellulose

Additional experimental data is required to determine if 6-deoxy-6-iodo-cellulose esters are better substrates for nucleophilic substitution reactions that 6-bromo-6-deoxy-cellulose esters. Due to time limitations, this data is not ready and available to be included in this dissertation. However, other researchers will be continuing this work, and it is expected that the data will be available in the near future.

Conclusions

Using reaction conditions previously used to produce iodinated cellulose derivatives from tosyl cellulose, 6-deoxy-6-iodo-cellulose ester derivatives have been synthesized from 6-bromo-6-deoxy-2,3-di-*O*-acyl-cellulose under Finkelstein reaction conditions. A high conversion of bromide to iodide was achieved, but the reactions were not completely quantitative as residual bromide was found in the products. The chemical structures of the products and regioselectivity of the reactions were confirmed by ^{13}C NMR. Since iodide is generally a better leaving group for

nucleophilic substitution reactions than bromide, the 6-deoxy-6-iodo-cellulose esters may be superior substrates for conversion to new functional cellulose derivatives. Work to test this hypothesis was begun using triethylamine and triethylphosphine as nucleophiles to synthesize polycationic cellulose derivatives, though time limitations have prevented this work from being completed at this time. In the process, however, the first evidence of cellulose derivatized with a phosphine was generated.

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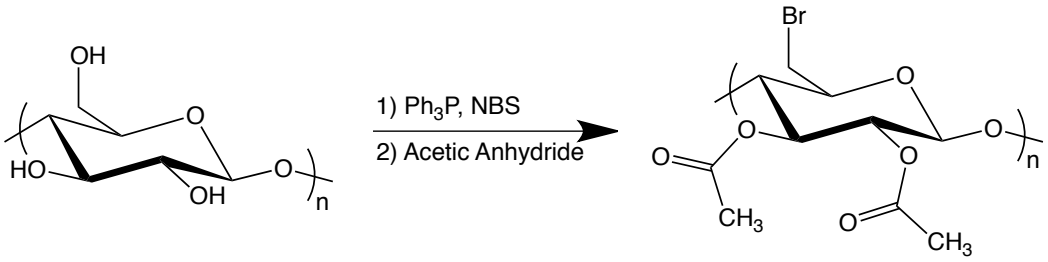
CHAPTER 7: SUMMARY AND FUTURE WORK

Esterification of Regioselectively Brominated Cellulose

Summary of results

An improved method has been devised for the regioselective "activation" of carbon 6 (*C*-6) on each anhydroglucose unit (AGU) in cellulose (Scheme 7.1). In this method, adapted from a procedure first published by Furuhata et al. (1992), the primary hydroxyl groups along the cellulose backbone are replaced by bromine by reaction with triphenylphosphine (Ph_3P) and *N*-bromosuccinimide (NBS) in a *N*,*N*-dimethylacetamide/lithium bromide (DMAc/LiBr) solvent system. The secondary hydroxyl groups are then esterified by the addition of a carboxylic anhydride to the reaction solution. The carbons at the *C*-6 position in the 6-bromo-6-deoxy-2,3-di-*O*-acyl-cellulose product can then be considered activated for nucleophilic substitution reactions since the bromide is a good leaving group and makes the carbon electrophilic. Thus, new functional groups can then be attached to the cellulose backbone at the *C*-6 position through substitution reactions. The high selectivity of the bromination reaction for *C*-6 on cellulose was first described by Furuhata et al. (1992), and it is further supported by the ^{13}C NMR spectra of the products obtained in this study. The esterification of the secondary hydroxyl groups results in 6-bromo-6-deoxy-cellulose esters that exhibit good solubility in organic solvents, including dimethyl sulfoxide, *N*,*N*-dimethylformamide, acetone, and chloroform. In contrast, the unesterified 6-bromo-6-deoxy-cellulose does not have significantly improved solubility over unmodified cellulose, and thus achieving high conversions in substitution reactions with brominated cellulose up till now required that the starting material undergo reactive dissolution (Saad, et al. 1996; Aoki, et al. 1999; Matsui, et al. 2005) or that it was first redissolved in DMAc/LiBr (Aoki, et al. 1996; Furuhata and Ikeda 1999). The good solubility of the esterified 6-bromo-6-deoxy-cellulose enables any subsequent reactions to be run under homogeneous conditions in solvents commonly found in an organic chemistry laboratory



Scheme 7.1 The one-pot synthesis of 6-bromo-6-deoxy-2,3-di-*O*-acetyl cellulose

The potential utility of the 6-bromo-6-deoxy-2,3-di-*O*-acyl-cellulose can be illustrated in a comparison to tosylated cellulose. The tosyl group is also a good leaving group for substitution chemistry, and therefore it has a similar activating effect for an adjacent carbon atom to that of bromine. The tosylation of cellulose also occurs preferentially at the primary hydroxyl groups attached to *C*-6, and tosyl cellulose has been used in many instances over the past couple of decades in the regioselective synthesis of functional cellulose derivatives. The reactions of nitrogen-based nucleophiles with tosyl cellulose in particular have been used to produce several different new cellulose derivatives designed for use in biosensor applications, hemocompatible coatings, and antimicrobial coatings (Fox, et al. 2011). However, the tosylation of cellulose is not as selective for the *C*-6 position as the bromination of cellulose. According to Rahn et al. (1996), a tosyl degree of substitution (DS) of approximately 1.4 is necessary before all the *C*-6 positions are substituted, meaning that a tosyl DS of 0.4 is distributed among the *C*-2 and *C*-3 positions. For this reason, it has been found in several cases that nucleophilic substitution on tosyl cellulose will occur at *C*-6 as well as *C*-2 and *C*-3, compromising the regioselectivity of the reactions. In the case of the 6-bromo-6-deoxy-cellulose esters, little or no bromide substitution is present at the *C*-2 and *C*-3, and therefore no substitution reactions can occur at those positions. Thus, many of the same cellulose derivatives that have been synthesized through tosyl cellulose intermediates can be synthesized with higher regioselectivity from 6-bromo-6-deoxy-cellulose.

Proposed future work

In their original paper on the bromination of cellulose, Furuhata et al. (1992) confirmed the high regioselectivity of the reaction by hydrolyzing the products in acid and analyzing *O*-trifluoroacetyl derivatives of the resulting glucose residues by gas chromatography. The analysis

did not detect any glucose residues with bromide substituted at *C*-2 or *C*-3. However, the synthesis of the 6-bromo-6-deoxy-cellulose esters reported here in Chapter 3 resulted in derivatives with a bromide DS greater than 1.0. It has already been shown that some of the *C*-6 positions in these derivatives have been substituted by acetyl groups (see Scheme 3.1), and possible bromide substitution at the end groups of the cellulose chain cannot account for the high bromide DS. Therefore, it appears likely that bromide substitution is occurring to a small extent at *C*-2 and *C*-3. ^{13}C NMR spectroscopy is not a sensitive enough technique to detect these substitutions. A chromatographic analysis of glucose residues from the 6-bromo-6-deoxy-cellulose esters, similar to that performed by Furuhata, should be sensitive enough to quantify any bromide substitution at *C*-2 or *C*-3. Such an analysis would be necessary for a full understanding of the substitution pattern resulting from the bromination reactions described in Chapter 3.

Microcrystalline cellulose was chosen as the starting material for the reactions reported here due to experimental convenience. The DMAc/LiBr solution viscosity is considerably lower with the relatively low molecular weight microcrystalline cellulose (MCC) than with higher molecular weight cellulose pulps or cotton. In their paper, Furuhata et al. (1992) briefly described the dissolution and bromination of absorbant cotton. They reported that 6 equivalents of the Ph_3P and NBS reagents in relation to the number of AGUs present in the reaction were required to get a high bromide DS with the higher molecular weight starting material. This is the only data found in the literature demonstrating the effect of the molecular weight of the cellulose on the bromination reaction, and more data on the topic is needed.

One significant limitation to the bromination reaction is that MCC cannot be dissolved in DMAc/LiBr in high concentrations, severely restricting the amount of 6-bromo-6-deoxy-cellulose that can be synthesized in a given batch. Furuhata et al. reported that a 3.3% (w/v) concentration of MCC dissolved in DMAc/LiBr solidifies below 70 °C. For the reactions in this research, a concentration 2.5% (w/v) of MCC in DMAc/LiBr was used. Other solvent systems should be investigated for this reaction to see if higher cellulose concentrations are achievable. An ionic liquid containing a bromide anion may be a good candidate if one can be found that is capable of dissolving both cellulose and the reactants. (1-Butyl-3-methylimidazolium bromide and 1-allyl-3-methylimidazolium bromide, two known cellulose solvents, do not dissolve Ph_3P.) Alternatively, a combination of an appropriate ionic liquid with an appropriate organic solvent

might provide a suitable solvent system. A third option would be to investigate the 1,3-dimethyl-2-imidazolidinone (DMI)/LiBr solvent system for the bromination of cellulose. Cellulose has successfully been dissolved in DMI/lithium chloride solvent systems (Yanagisawa and Isogai 2005), though there are no reports of attempting to dissolve cellulose in DMI/LiBr. An additional potential benefit to finding alternative solvents for this reaction is that the acetylation side reaction at *C*-6 might be avoided (see Scheme 3.1).

Attempted Synthesis Regioselectively Carboxylated Cellulose Derivatives

Summary of results

Attempts were made to synthesize regioselectively carboxylated cellulose derivatives from tosyl cellulose and 6-bromo-6-deoxy-cellulose esters. Our interest in making these derivatives stemmed from the frequent inclusion of carboxylated cellulose in oral drug formulations to help control the release and improve the bioavailability of a drug in the gastrointestinal tract (Edgar 2007). Carboxylic acid groups attached to the cellulose can both serve to improve the affinity of the drug for the cellulosic polymer matrix and impart pH-responsive properties to the polymer useful for controlled release. However, all of the carboxylated cellulose derivatives that are commercially available for these applications are synthesized using traditional cellulose reactions that result in a near random distribution of the carboxylic acid substituents between *C*-2, *C*-3, and *C*-6. Our intention was to devise a method to selectively attach carboxylic acid groups at *C*-6 and use the resulting derivatives to determine if this selectivity had an effect on the drug releasing properties of the polymers.

Unfortunately, the attempts to synthesize the new carboxylated cellulose derivatives were not successful. In the first set of reactions, iodinated tosyl cellulose was dissolved in a solution with a rhodium carbonyl catalyst and subjected to high carbon monoxide pressure and high temperature. The reaction conditions mimicked the conditions used for the industrial synthesis of acetic acid and acetic anhydride from methanol and methyl acetate, respectively (Zoeller, et al. 1992; Haynes 2006). However, analysis of the products indicated that the polysaccharide structure had been degraded, and the reaction was abandoned in favor of a different approach. The new attempt involved the reaction of 6-bromo-6-deoxy-2,3-di-*O*-acyl-cellulose with sodium cyanide to synthesize 6-cyano-6-deoxy-cellulose esters. The nitrile then was to be hydrolyzed at

high pH to form a carboxylic acid. Synthesis of the cellulose nitrile derivative was successful, providing a demonstration of the utility of the brominated cellulose esters as a substrate for nucleophilic displacement reactions, though molecular weight analysis of the products indicated that mild degradation of the polysaccharide chain had occurred. Attempts at the base catalyzed hydrolysis of the nitrile, however, resulted in the complete degradation of the polysaccharide. It was proposed that the protons alpha to the nitrile are susceptible to abstraction by the base, which could result in a chain reaction that causes the cleavage of the polysaccharide chain (see Scheme 4.3).

Proposed future work

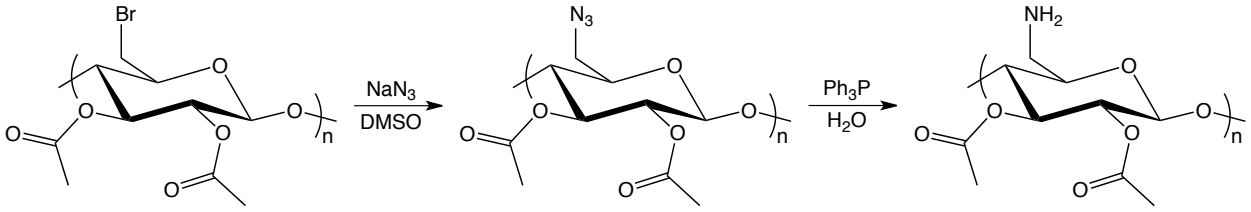
A considerable amount of effort was expended trying to produce carboxylated cellulose derivatives using the methods described here. It would seem unwise at this point to continue such efforts to get them to work as desired unless a wholly original approach is devised. However, nitriles can also be converted to amines, and thus the 6-cyano-6-deoxy-2,3-di-*O*-acyl-cellulose derivatives reported here could also be used as precursors to new regioselectively aminated cellulose derivatives. Such derivatives would be similar to those described in Chapter 5 but with one additional carbon atom between *C*-6 and the nitrogen. Lithium aluminum hydride is often the reducing agent used to convert nitriles to amines, but sodium acetoxyborohydride might be a more suitable choice in this instance since it will preferentially reduce nitriles in the presence of esters (Gribble 1998). It would be interesting to compare the solubility properties of the amino cellulose esters prepared from the reduction of 6-cyano-6-deoxy-cellulose esters versus the amino cellulose esters prepared from the reduction of 6-azido-6-deoxy-cellulose esters described in Chapter 5.

Synthesis of 6-Amino-6-Deoxy-2,3-di-*O*-Acyl-Cellulose

Summary of results

In a further demonstration of the utility of the 6-bromo-6-deoxy-2,3-di-*O*-acyl-cellulose as a substrate for nucleophilic substitution reactions, it was reacted with sodium azide to displace the bromide and form the corresponding 6-azido-6-deoxy-cellulose esters. The azide was then

selectively reduced to a primary amine under mild Staudinger reaction conditions (Scheme 7.2). The structure of the azide derivative was confirmed by ^{13}C NMR and FTIR analysis. While the azide substitution reaction had been previously reported with both tosyl cellulose and non-esterified 6-bromo-6-deoxy-cellulose (Liu and Baumann 2002; Matsui, et al. 2005; Heinze, et al. 2006), the reaction reported here demonstrated several advantages. Azide substitution on tosyl cellulose was found to occur at *C*-2 and *C*-3 at reaction temperatures higher than 50 °C due to the presence of tosyl groups at those positions on the substrate. Selective substitution at *C*-6 could be achieved at 50 °C, though the reaction time had to be extended to 38 h for complete conversion to take place. On the other hand, azide substitution on non-esterified 6-bromo-6-deoxy-cellulose could be run at higher temperature since none of the *C*-2 and *C*-3 positions were susceptible to the reaction, though a long reaction time of 48 h was required for complete conversion due to the lack of solubility of the starting material in the reaction solvent. In the reactions reported here, selective and high conversion of the bromide to the azide was possible at 80 °C in 24 h.



Scheme 7.2 The synthesis of 6-amino-6-deoxy-2,3-di-*O*-acetyl-cellulose, starting from 6-bromo-6-deoxy-2,3-di-*O*-acetyl-cellulose

The reduction of the azide to the amine using Ph_3P reported here was, to our knowledge, the first time that the Staudinger reduction has been used on a polysaccharide substrate. The reaction was demonstrated at room temperature in both tetrahydrofuran and DMAc. Monitoring the reaction by *in situ* FTIR indicated that it had finished in 8 h, and analysis of the isolated product showed that nearly all the azide groups had been reduced. One issue encountered with the reaction was that acyl migration from the *C*-2 and *C*-3 hydroxyl substituents to the amino group at *C*-6 appeared to occur quite readily. It was found that this side reaction was more prevalent when the Ph_3P was added to the reaction solution under anhydrous conditions, and it could be minimized by introducing water at the start of the reaction. This is likely due to the

reactivity of the iminophosphorane intermediate that forms after the reaction of the Ph_3P with the azide followed by the splitting off of nitrogen gas (see Scheme 5.2). The iminophosphorane is highly susceptible to attack by an acylating reagent, resulting in the formation of an amide instead of a free amine. The presence of water causes the iminophosphorane to be quickly hydrolyzed to the less reactive amine before the acyl migration reaction can occur.

While the reaction did proceed entirely under homogeneous conditions, an effective solvent for the isolated 6-amino-6-deoxy-2,3-di-*O*-acyl-cellulose could not be found. This issue was overcome by taking advantage of the reactivity of the iminophosphorane. By running the reaction under anhydrous conditions in the presence of a carboxylic anhydride, a soluble product that was fully *O*- and *N*-acylated was produced. A different acyl group could be attached to *C*-6 through an amide bond than that which was already attached to *C*-2 and *C*-3 through ester bond by choosing an appropriate carboxylic anhydride for the reaction. In this manner, a new method for the regioselective acylation of cellulose has been created.

Proposed future work

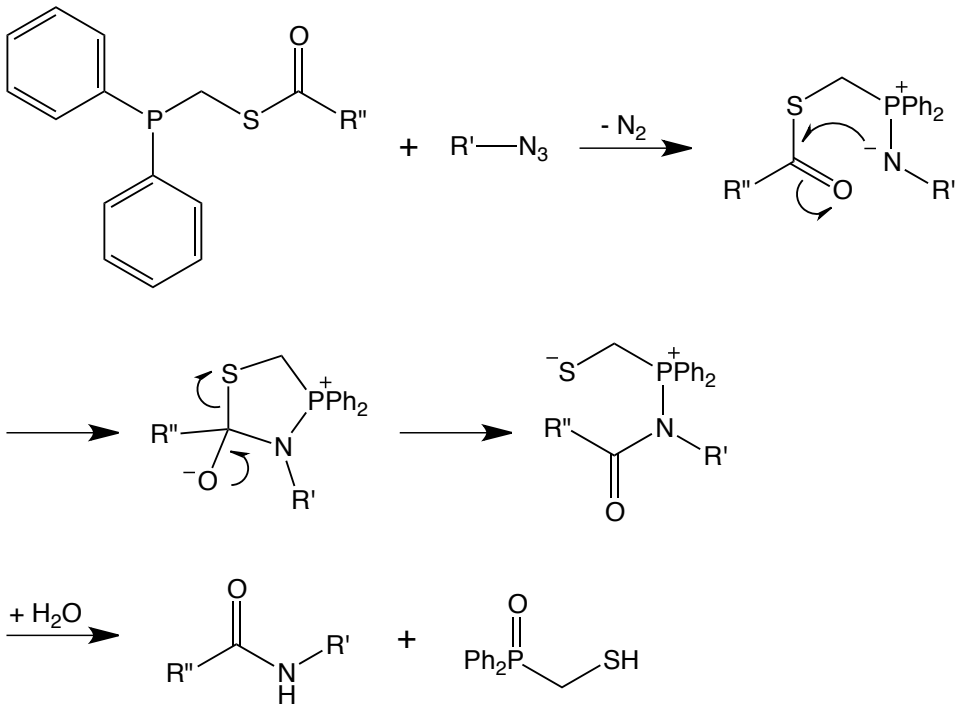
The 6-amino and 6-amido-6-deoxy-cellulose esters could potentially have interesting properties for oral drug delivery. The lack of solubility of the 6-amino-6-deoxy-cellulose esters would inhibit their use in true amorphous matrix formulations, but physical mixtures of the compounds with anionic drugs could potentially be used to extend the release of the drug. Physical mixtures of carboxymethylcellulose acetate butyrate (CMCAB) and aspirin, ibuprofen, or fexofenadine HCl are similar examples that have previously been shown to result in extended drug release (Posey-Dowty, et al. 2007). Since the 6-amido-6-deoxy-cellulose esters do readily dissolve in organic solvents, they could be tested for their effectiveness as the matrix material for amorphous solid dispersions of different drugs. There has been little published research on the use of amide containing polymers for this purpose, even though the ability of amides to form hydrogen bonds might promote interactions between a drug and the polymer. The 6-amido-6-deoxy-cellulose esters could help fill in that gap in the literature.

The demonstrated effectiveness of the Staudinger reaction in selectively reducing azide groups attached to cellulose to amines opens interesting new possibilities for the synthesis of other new cellulose derivatives. One possibility that could be explored is the use of the “traceless” Staudinger ligation to regioselectively attach a wide variety of functional groups to cellulose

through an amide bond at *C*-6 (Saxon, et al. 2000). The traceless Staudinger ligation is a variation on the Staudinger reaction in which a carbonyl group is attached to the phosphine reducing agent through a cleavable linkage. This reaction has been demonstrated with several reagents, but one that is commonly used for this purpose is (diphenylphosphino)methanethiol (Soellner, et al. 2006). The general reaction mechanism for the traceless Staudinger ligation using this reagent is illustrated in Scheme 7.3. The functional group (R″) that is eventually to be attached to the azide compound through an amide bond is first bonded to the (diphenylphosphino)methanethiol through a thioester bond. The azide and the phosphine react to form a phosphazide, which loses nitrogen gas to form an iminophosphorane as in the traditional Staudinger reaction. The nitrogen in the iminophosphorane then undergoes an intramolecular nucleophilic attack on the carbonyl carbon of the thioester. The carbon to sulfur bond is then cleaved, and an amide is formed. The phosphine is next hydrolyzed from the nitrogen, forming the phosphine oxide and the free amide. In this scheme, one can easily imagine the starting azide compound as a 6-azido-6-deoxy-cellulose ester and R″ as a variety of functional groups or compounds. Since it was demonstrated in Chapter 5 that the iminophosphorane intermediate formed during the reduction of the cellulose azide was readily reactive with ester groups on adjacent cellulose chains, there is a high probability that the same iminophosphorane intermediate would also be reactive with a thioester. If the reaction is indeed successful, the traceless Staudinger ligation could be a powerful tool for regioselectively attaching a range of functional groups to a cellulose and other polysaccharides.

Another potentially interesting variation on the Staudinger reaction with 6-azido-6-deoxy-cellulose esters would be to react the iminophosphorane intermediates with different aryl or alkyl chloroformates to form a carbamate bond at *C*-6. This reaction has already been demonstrated to proceed readily on low molecular weight model compounds (Ariza, et al. 1999). Carbamates are frequently used as protecting groups for amines in amino acids since different carbamates can easily be removed to regenerate the amines under a variety of reaction conditions. By choosing an appropriate chloroformate to react with the cellulose azide, the carbamate bond formed can later be cleaved to generate the 6-amino-6-deoxy-cellulose ester without affecting the ester bonds at *C*-2 and *C*-3. For example, the benzyl carbamate can be hydrogenated using palladium hydroxide as a catalyst to form the amine (Wuts and Greene 2006). One potential advantage of this chemistry is that the 6-carbamate-6-deoxy-cellulose ester might possess better

solubility in organic solvents, which would allow it to be formed into films or particles that could then be deprotected to produce the 6-amino-6-deoxy-cellulose esters.



Scheme 7.3 The general reaction mechanism for the traceless Staudinger ligation with a (diphenylphosphino)methanethioester as the reducing agent (Soellner, et al. 2006)

Synthesis Regioselectively Iodinated Cellulose

Summary of results

Regioselectively iodinated cellulose esters were synthesized by reacting 6-bromo-6-deoxy-2,3-di-*O*-acyl-cellulose with sodium iodide under Finkelstein reaction conditions. An iodide DS of up to 0.95 was achieved, though a residual bromide DS of between 0.09 and 0.14 was measured for all of the samples produced. The reactions resulted in high yields and little to no degradation of the polysaccharide chain. This method represents an improvement over the previously reported method of reacting cellulose tosylates with sodium iodide under the same reaction conditions (Rahn, et al. 1996). As discussed above, the brominated cellulose is a more regioselectively substituted derivative than tosyl cellulose, which has significant occurrence of

tosyl groups at the *C*-2 and *C*-3 positions. This gives the iodide the opportunity to react with the *C*-2 and *C*-3 positions on tosyl cellulose, and indeed the presence of iodide at *C*-2 can be detected in the ^{13}C NMR spectrum of iodinated tosyl cellulose (see Figure 4.3). A study by Liu and Baumann (2002) found that the displacement of tosylates by iodide on tosyl cellulose could be made selective for *C*-6 by lowering the reaction temperature from 120 °C to 60 °C, though the reaction time had to be extended from 2 h to 42 h for high conversion to be achieved. With the brominated cellulose, few if any bromide substituents are found at the *C*-2 and *C*-3 positions, and therefore the chance of an iodide reacting at those positions is minimized. Thus, the iodination reaction can be run at the higher temperature to achieve high conversions in 2 h and still retain its high regioselectivity.

The practical advantage to converting the 6-bromo-6-deoxy-cellulose esters to 6-deoxy-6-iodo-cellulose esters is that the iodide is generally a better leaving group for substitution reactions than the bromide. To date, however, there have been no reports in the literature of regioselectively iodinated cellulose being used as substrates for nucleophilic displacement reactions. Here, the first reactions with iodinated cellulose were run with triethylamine and triethylphosphine as nucleophiles. The former was previously used in reactions with tosyl cellulose to produce water soluble polycationic cellulose derivatives, though only low substitution of the quaternized amine was achieved (Koschella and Heinze 2001). There have been no previous reports on the synthesis of phosphine substituted cellulose derivatives. NMR spectra of the products from the reactions with both nucleophiles indicated that some conversion did take place, though more characterization of these derivatives is needed. More data is also required for any conclusions on the relative suitability of iodinated cellulose derivatives for substitution reaction in relation to brominated or tosylated cellulose.

Proposed future work

The reaction of 6-bromo-6-deoxy-cellulose esters with sodium iodide needs to be studied further to see if varying the reaction conditions, such as time or reagent ratios, will result in the complete displacement of bromide with iodide. The regioselectivity of the reaction could be examined in more detail by acid hydrolysis of the cellulose derivatives and chromatographic analysis of the glucose residues as proposed above for the 6-bromo-6-deoxy-cellulose esters.

Since iodide is known to displace tosylates at *C*-2 and *C*-3 on cellulose under high temperatures, presumably it can also displace bromides that might be present at those positions.

As mentioned above, more data is necessary to compare the leaving group abilities of iodide and bromide in substitution reactions with cellulose. Kinetic analysis of the reaction of triethylamine and either 6-bromo-6-deoxy-2,3-di-*O*-acetyl-cellulose or 6-deoxy-6-iodo-2,3-di-*O*-acetyl-cellulose should be performed by removal of aliquots of the reaction solution at given time intervals and isolation of the products. Other weak nucleophiles such as carboxylates may be suitable for this analysis as well. However, several attempts over the course of this work to react sodium acetate or silver acetate with 6-deoxy-6-iodo-2,3-di-*O*-propionyl-cellulose were not successful at producing the *C*-6 acylated product.

The reaction of 6-deoxy-6-iodo-2,3-di-*O*-acetyl-cellulose with triethylphosphine appears to be a promising method for the synthesis of polycationic derivatives of cellulose. More work is needed to optimize the reaction conditions and figure out the best isolation method for the product. Additional characterization of the product is also needed to confirm the structure of the derivative, determine the DS of the phosphonium substituent, and determine if the reaction results in any molecular weight loss of the polysaccharide.

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