Clickable Polymers Accessible through Nucleophilic Substitution on Polysaccharides: A Sophisticated Route to Functional Polymers

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This review article is dedicated to special polysaccharide esters – the polysaccharide toluenesulfonic acid esters (tosylates) and polysaccharide carbonate esters. After describing the specifics of the synthesis, particular emphasis is placed on the use of polysaccharide tosylates and polysaccharide phenyl carbonates for subsequent modification by nucleophilic substitution (S_N) reactions. For this purpose, the advantages and limitations of the respective derivatives are discussed with regard to their application in chemical modification with nucleophiles containing functional groups. A few functional polysaccharide derivatives and their properties are presented. Finally, reactive derivatives for click chemistry approaches are featured. These can be prepared starting from the reactive intermediate of either polysaccharide tosylate or polysaccharide phenyl carbonate.

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INTRODUCTION

The chemical modification of polysaccharides remains the most important tool to obtain products with application-specific properties. Cellulose, as the most frequently used polysaccharide, offers access to broad variety commercial derivatives that are produced on a large scale. Cellulose ethers are produced by the Williamson ether synthesis with alkyl halides such as methyl- and ethyl chloride as well as by ring-opening reaction of ethylene- and propylene oxide as reagents. Prior to etherification, cellulose must be activated, which is done exclusively with aqueous sodium hydroxide in a concentration range from 15 to 50%. The reaction occurs under heterogeneous conditions. Both the aqueous alkaline and heterogeneous conditions are reasons for the limited number of commercially available cellulose ether products. Most of the cellulose ethers are water-soluble and applied in aqueous systems, as discussed in detail by Wuestenberg (2013).

Another important group of commercial cellulose derivatives is esters of carboxylic acids. Regarding the carboxylic acid ester of cellulose, the number of different products is also limited. The acetate, acetate propionate, acetate butyrate, and the acetate phthalate of cellulose are industrially manufactured.

Due to the conditions of esterification on a technical scale, carboxylic acids with more than 4 C atoms do not react efficiently with the biopolymer. These cellulose esters are soluble in organic solvents and find widespread applications (Edgar *et al.* 2001). Despite these commercial products from cellulose, it is important to develop new methods for chemical modification. With the increasing complexity of applications, especially in the biomedical context, the requirements for the synthesis of polysaccharide derivatives are also becoming more demanding. The robust synthesis of products should be feasible, selective, and easily adaptable by changing parameters such as solvent and functionality.

Although the physico-chemical properties of the polysaccharides are different, the modification can be adapted to other backbones of neutral polysaccharides carrying hydroxyl groups (Cumpstey 2013). This is particularly important to ensure that the requirements of applicability are achieved and that the range of diversity in structure and source of the polysaccharides is exploited. Bacterial dextran, which is popular in the biomedical field, but also other neutral polysaccharides, such as pullulan from fungi or pure xylan from wood, can be attractive starting materials. Chemical modification of polysaccharides based on click chemistry can be such a strategy as highlighted in the review of Meng and Edgar (2016). Although some derivatives already exist, it is still important to better implement the diversity of these reactions in the field of polysaccharides.

Special cases of previous mentioned esters of polysaccharides are sulfonic acid esters and carbonate esters of cellulose, which are not applied directly as commercial products, but are used as a platform for the synthesis of subsequent products (Trivedi and Fardim 2019).

The main part of the feature article will deal with the use of, in particular, *p*-toluene sulfonate (tosylate) and phenyl carbonate, as a reactive platform and the different opportunities of nucleophilic substitution (S_N) reactions with nucleophiles (inverse reactivity than hydroxyl group), the products accessible, their properties, and applications. The advantages (achievable regioselectivity, complete conversion or derivatives with mixed reactive moieties) and disadvantages (*e.g.*, limited reactivity) of each reactive platform are discussed. Some examples of "clickable" derivatives, starting from cellulose and extended to other polysaccharides, obtained by the S_N reactions will also be highlighted. It must be mentioned that many issues discussed resulted from the authors' own studies.

POLYSACCHARIDE *p*-TOLUENE SULFONIC ACID ESTER (POLYSACCHARIDE TOSYLATE)

Synthesis of Polysaccharide tosylates

Although the synthesis of cellulose tosylates (Fig. 1) was first realized heterogeneously by reaction of cellulose with *p*-toluene sulfonic acid chlorides (tosyl chloride) in aqueous alkaline media (NaOH, Schotten-Baumann reaction), homogeneous procedures are preferred to obtain pure products with controlled degree of substitution (DS) and distribution of substituents (Heinze *et al.* 2006a).

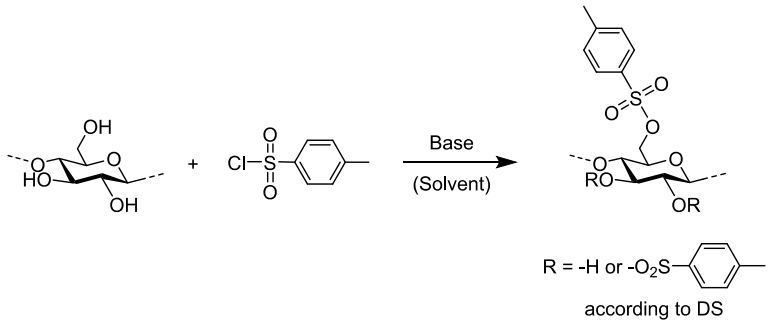

Fig. 1. Reaction scheme for homogeneous tosylation of cellulose

Thus, the synthesis of cellulose tosylate is carried out by reacting the biopolymer with tosyl chloride, applying the efficient cellulose solvent *N*,*N*-dimethylacetamide (DMAc) in combination with LiCl. As early as 1986, McCormick and Callais prepared pure cellulose tosylate (McCormick and Callais 1987). As summarized in Table 1, products with a DS as high as 2.4 could be obtained. In other systematic studies, it turned out that the reaction efficiency decreases with increasing degree of polymerization (DP) of the cellulose sample (cellulose powder, DP 280; spruce sulfite pulp, DP 330 and 650; cotton linters, DP 850; beech sulfite pulp, DP 1020, and bacterial cellulose, DP 5100). Moreover, the DS depends on the molar ratio cellulose to tosyl chloride. The highest DS value reached was 2.3, by applying pulp with DP of 330 and a molar excess of 4.5 moles tosyl chloride (Rahn *et al.* 1996). A complete tosylation of the cellulose was not possible even at higher molar excess of reagent. The reason for this upper limit DS of about 2.4 is still unknown. Unlike other sulfonates like mesylate, brosylate, and triflate, the polysaccharide tosylate is a very stable derivative (Siegmund and Klemm 2002) and the limitation of the DS could be attributed to the size of the sulfonate and the associated steric hindrance at the polysaccharide coil. In addition, a base must be applied in the tosylation reaction to bind the hydrogen chloride formed during the reaction. Pyridine was used in previous studies, and it leads to side reactions, mainly to the formation of dexoychloro and acetate moieties. The most appropriate base is trimethylamine to exclude this side reaction and to apply reaction temperature up to room temperature.

Recently, other solvents were studied as reaction media for the homogeneous tosylation of cellulose. Aqueous sodium hydroxide (Elchinger *et al.* 2012) and NaOH-urea aqueous system (Schmidt *et al.* 2014) were applied as "green" solvents. The tosyl cellulose was capable of being obtained. Compared to the reaction in the water-free DMAc/LiCl solvent, a significantly higher molar excess of reagent to cellulose is needed to achieve comparable DS values because of the partial hydrolysis of the tosyl chloride in the aqueous systems.

Ionic liquids (ILs) are powerful solvents for cellulose (El Seoud *et al.* 2007). The tosylation of cellulose in 1-allyl-3-methylimidazolium chloride (AMIM Cl) in the presence of pyridine as a base at 10 °C yields no reproducible results (Granström *et al.* 2008). The disadvantage of this solvent is its high viscosity, which leads to insufficient stirring and mixing of the reaction mixture, so that the products tend to be similar to those of heterogeneous reactions. Applying AMIM Cl or 1-ethyl-3-methylimidazolium diethyl phosphate (EMIM DEP) in combination with pyridine or organic solvents, such as N,N-dimethylformamide (DMF) or 1,3-dimethyl-2-imidazolidinoneem, leads to homogeneous conditions and consequently to tosylation predominantly at the primary hydroxyl group of

C6 (Gericke *et al.* 2012). The organic co-solvent decreases the viscosity of the reaction systems significantly and guarantees a mixing of the reaction components. These synthesis paths are also not preferred due to the high price of the ILs and the lack of sufficient recycling concept for ILs. It was unexpected that the conversion of cellulose dissolved in 1-ethyl-3-methylimidazolium acetate (EMIM Ac) with tosyl chloride resulted in the formation of pure cellulose acetate. This result is explained by a side reaction of the IL solvent EMIM Ac with tosyl chloride, forming the mixed acetic acid-toluene sulfonic anhydride, which was demonstrated by nuclear magnetic resonance (NMR) spectroscopic studies (Köhler *et al.* 2007). Subsequent reaction of cellulose with the mixed anhydride occurs on the acetyl moiety. Therefore, ILs with less nucleophilic anions must generally be used in the reaction of acid chlorides.

Table 1. Overview of Solvent/Base Systems for Tosylation of Cellulose

Solvent	Base	DS	Literature
DMAc/LiCl	Triethylamine	2.4	McCormick and Callais 1987
DMAc/LiCl	Triethylamine	2.3	Rahn *et al.* 1996
$NaOH_{aq.}$	-	1.7	Elchinger *et al.* 2012
Ionic liquids + (co-solvent)	Pyridine or triethylamine	1.1	Gericke *et al.* 2012
$NaOH_{aq.}$/urea	-	1.7	Heinze *et al.* 2014
Pyridine*	-	0.9	Arai and Aoki 1994
* Preactivation with $NaOH_{aq.}$ (mercerization)			

The homogeneous synthesis of tosylate in DMAc/LiCl can be transferred to other polysaccharides in addition to the most commonly used cellulose. The preparation of tosylates was investigated for polysaccharides as α-1,4-linked starch (Dicke *et al.* 2001), pullulan (α-1,4; α-1,4; α-1,6), dextran (α-1,6), lichenan (β-1,4 and β-1,3) (Koschella *et al.* 2006), and other 1,3-linked glucans (Koschella *et al.* 2020a), as well as agarose (Gericke and Heinze 2015). Although the efficiency of the reaction is sufficient for these polysaccharides and the efficiency is often comparable to the conversion with cellulose, the regioselectivity observed differs for the polysaccharides. It was found that the regioselectivity is connected to the type of glycosidic linkage of the polysaccharide. For cellulose the reactivity of hydroxyl group 6>>2>3 occurs. For agarose, the primary hydroxyl groups possess also the highest reactivity with tosyl chloride. In contrast, starch reacts preferentially at position 2. The α-1,3-linked glucan also shows this reactivity, while the preference is not as pronounced as with starch. Moreover, dextran, which consists predominantly of a main chain of α-1,6-linked glucose, tosylation could be realized. However, the product is not well appropriate for S_N reactions (see limitations).

The synthesis of polysaccharide tosylates is well explored and there is not much scope for optimization. A more sustainable aqueous medium leads to lower efficiency and therefore does not hold promise. The conditions have been extensively studied, and mild conditions, such as low temperatures, have a positive effect on the efficiency of the reaction and reduce side reactions. By upscaling the process, the amount of solvent DMAc/LiCl can be reduced without significantly decreasing the DS values, while keeping the processing manageable. The solvent for the isolation of the product by precipitation can be recycled to a large extent.

NUCLEOPHILIC DISPLACEMENT REACTIONS WITH POLYSACCHARIDE TOSYLATE

Advantages

An advantage of the introduction of tosylate groups into the polysaccharide backbone is the improvement of solubility in organic solvents, especially for cellulose, which is insoluble in conventional solvents in its unmodified form (Koschella *et al.* 2006). The stability of the tosyl group is considered as a further advantage; it is stable for further modification of the remaining hydroxyl groups before the reactive group is reacted with nucleophiles such as azide or amines (Fig. 2). To modify the properties of cellulose-*p*-toluene sulfonic acid ester and hence of the final product obtained by S_N reaction, the remaining hydroxyl groups of cellulose tosylate may be modified prior to the S_N reaction. The tosylate groups are stable under the usual conditions of esterification. Thus, cellulose tosylates were converted into mixed cellulose tosylate/carboxylates (Heinze *et al*. 1996), tosylate/urethanes (Tiller *et al*. 2001), and tosylate/sulfate (Heinze and Rahn 1996). Even a complete functionalization of all remaining hydroxyl groups was achieved with acetic- and propionic acid anhydride. The products are readily soluble in dipolar aprotic solvents, such as acetone, dimethyl sulfoxide (DMSO), N,N-dimethylacetaminde (DMAc), and tetrahydrofuran (THF), and can be cast as films from solution. Mixed cellulose esters that carry the tosylate and monoester of a dicarboxylic acid (*e.g.*, tosylate/phthalate or succinate) are soluble in aqueous NaOH and even in water, depending on the ratio of the DS values. Cellulose tosylate sulfate shows self-aggregation in water due to the amphiphilic character of the derivative (Rahn *et al.* 1996). The hydrophobic character of the tosylate in combination with the repulsive electrostatic force of the sulfate leads to aggregation in aqueous media.

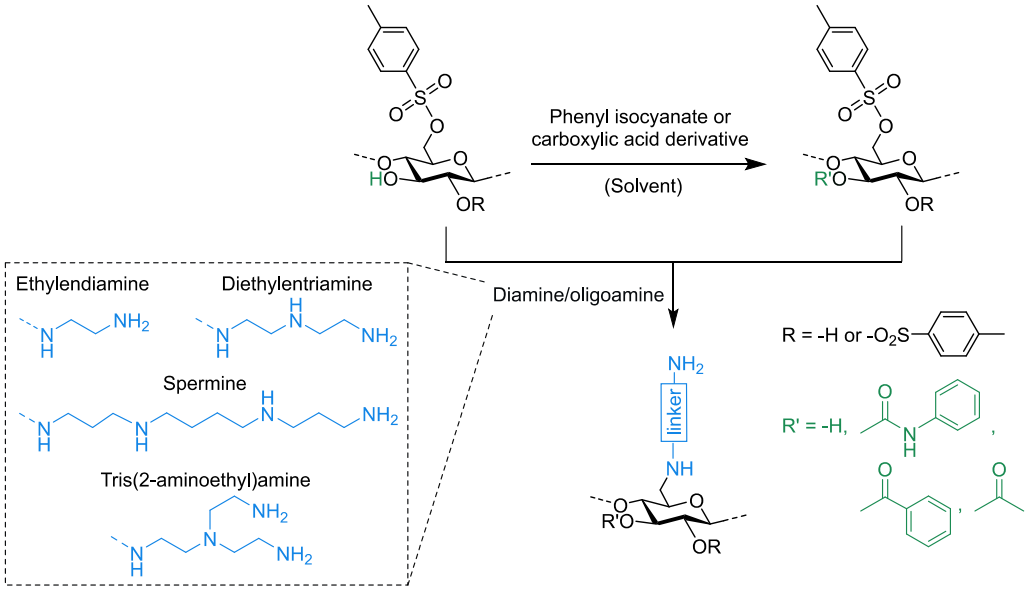

Fig. 2. Chemical modification of cellulose tosylate and preparation of different aminocelluloses

The main advantage of polysaccharide tosylates is the high reactivity with nucleophiles. Nucleophiles are bound to the polymer backbone by nucleophilic

substitution. For example, aminolysis works well for cellulose, whereby aminocellulose with terminal amino groups can be obtained without protection and side reactions (Fig. 2). As the structurally simplest compound (Fig. 2, without linker), hydrazine hydrate can be taken as nucleophile (Nazir *et al.* 2021). Formally, there is also a terminal NH_2 group in the product, although its reactivity is not the same as that of the diamines. Even tertiary diamines react sufficient with the tosylate of the primary hydroxyl group of cellulose and also asymmetric diamines can be applied for synthesis. The terminal tertiary amino moiety of the obtained derivative could be further converted in a ring-opening reaction to yield sulfobetainic polyampholytes based on cellulose (Pfeifer *et al.* 2022). For cellulose, a regioselective functionalization at the primary hydroxyl group at C6 is achievable up to a DS of 0.8. Consequently, it is possible to substitute all the tosylate groups by nucleophiles such as sodium azide or functional amines, forming a product with a defined structure and substitution pattern. Even pure 6-deoxyazido- or 6-deoxyamino agarose up to a DS of 0.6 could be synthesized by this route using sodium azide or ethylenediamine as nucleophile (Gericke and Heinze 2015). The conversion of polysaccharide tosylates with sodium azide was completed not only for cellulose (see section clickable polysaccharide derivatives), but also for dextran-, pullulan-, and lichenan tosylates, yielding well soluble samples of mixed tosylate-deoxyazido derivatives that are appropriate for the click chemistry approach.

Limitations

In the synthesis of polysaccharide tosylate, halogenation of the hydroxyl group is a possible side reaction. However, the reaction conditions of the homogeneous reaction in DMAc/LiCl can be controlled to avoid the deoxychloro group as a by-product. Thus, it is possible to obtain a pure polysaccharide tosylate as a reactive starting derivative for nucleophilic substitution reactions. The tosylation of various polysaccharides such as dextran, pullulan, lichenin, starch, and other glucans is well possible in DMAc/LiCl (see section synthesis of polysaccharide tosylates). However, they can only be used to a limited extent for subsequent chemical modification by nucleophilic displacement reactions. The highest rate of nucleophilic substitution reaction is observed for the tosylate of the primary hydroxyl group. For the conversion of dextran tosylate, which has no primary hydroxyl group in the main chain, a low DS of deoxyazido of 0.6 with a remaining DS of 0.7 could be synthesized. Up to now, the selective cleavage of remaining tosylate groups of a deoxyamino- or deoxyazido derivative is not reported.

CELLULOSE CARBONATES

Synthesis of Polysaccharide Carbonates

Heterogeneous conversion of cellulose is again less appropriate for the cellulose carbonate synthesis; for instance, the conversion of cellulose with ethyl chloroformate in DMSO (a non-solvent for cellulose) leads to non-uniform products containing both the trans-2,3-cyclic carbonate structure and the acyclic *O*-ethoxycarbonyl moiety (Barker *et al.* 1997).

In contrast, homogeneous reaction in DMA/LiCl yields the corresponding acyclic cellulose carbonates as uniform and soluble products with defined DS values. For the esterification of cellulose in DMA/LiCl, chloroformates and pyridine as base are most suitable (Elschner *et al.* 2013a). Applying an equimolar amount of repeating unit to

phenylchloroformate, a DS of 0.84 was achieved. Increasing the amount of phenyl chloroformate to 10 equivalents resulted in an increase in DS to 1.98. Although electronic aspects (smaller +I effect) should favor a reaction of the primary hydroxyl groups, this regioselective reactivity cannot be observed. In the solvent DMAc/LiCl highly functionalized cellulose phenyl carbonates are difficult to synthesize and require high molar ratios because chloroformate reacts with DMAc and is thereby consumed.

The reaction of cellulose with *p*-NO_2-phenyl chloroformate (molar ratio of 1:3) yields a product with a DS value of 1.34, which shows gel formation due to the crosslinking by intermolecular carbonate moieties. A comparable crosslinking was observed for cellulose phenyl carbonate with low DS (molar ratio 1:0.5). The crosslinking is based on primary hydroxyl groups due to the smaller +I effect and hence the increased reactivity of the carbonyl group, compared to carbonates derived from secondary hydroxyl groups. Thus, the remaining hydroxyl groups of cellulose may react despite their low nucleophilicity. The crosslinking of cellulose phenyl carbonates with low DS is a result of the high number of non-derivatized hydroxyl groups that are accessible for intra- and intermolecular carbonate formation. This hypothesis was proven by conversion of 6-*O*-trityl cellulose (Ganske and Heinze 2018). In this context, it is interesting to note that xylan, a hemicellulose consisting of β-1,4-glycosidically linked xylose, does not show any crosslinking during the formation of the corresponding phenyl carbonates independent of the DS. Both secondary hydroxyl groups present in the biopolymer could be converted with phenyl chloroformate either in dipolar aprotic solvents (DMAc, NMP) with LiCl or in an ionic liquid to products with DS of up to 2 (Fig. 3 structure characterization; Fig. 8 reaction scheme). Further, these xylan phenyl carbonates with high DS could be processed into stable reactive nanoparticles by precipitation approaches (Fig. 3). Unexpectedly, the hydroxyl group at position 3 is the most reactive, while substitution in position 2 only occurred if the neighboring position 3 is already modified (Gericke *et al.* 2018). The reason for this special reactivity is still unknown. Additionally, for the polysaccharides (starch, pullulan, and dextran), high DS values up 2.0 and 2.9 can be obtained (Elschner *et al.* 2013a).

Ionic liquids are efficient reaction media for the carbonate synthesis (Fig. 8, reaction of cellulose) because they are inert, *i.e.*, they do not react with rather reactive chloroformates. Cellulose, dissolved in 1-butyl-3-methylimidazolium chloride (BMIM Cl) and diluted with pyridine to decrease the viscosity of the system, was allowed to react with phenyl chloroformate at different molar ratios, yielding cellulose phenyl carbonates with controlled DS and even complete functionalization (DS = 3). The homopolymer gave well-resolved NMR spectra. Under mild reaction conditions no biopolymer degradation occurred. A DS of 2.83 at a molar ratio of 3:1 could be obtained using the IL as reaction medium, while the DS was only 1.49 when applying DMA/LiCl as solvent. The efficiency of the conversion increased from 50 to 94%, *i.e.*, the conversion occured almost quantitatively. The molar ratio of 5:1 yields a sample with DS of 3 whereas the DS is 1.75 in case of DMA/LiCl. Even at high molar ratios (10:1), the DS of 1.98 only could be realized in the tertiary amide solvent (Elschner *et al.* 2013b). Thus, exchanging the polar aprotic solvent by the ionic liquid BMIMCl is a good choice as a solvent to optimise polysaccharide-phenyl carbonate synthesis. The efficiency of the process (especially for high DS values/ complete modification) can be increased and the reagents and by-products reduced.

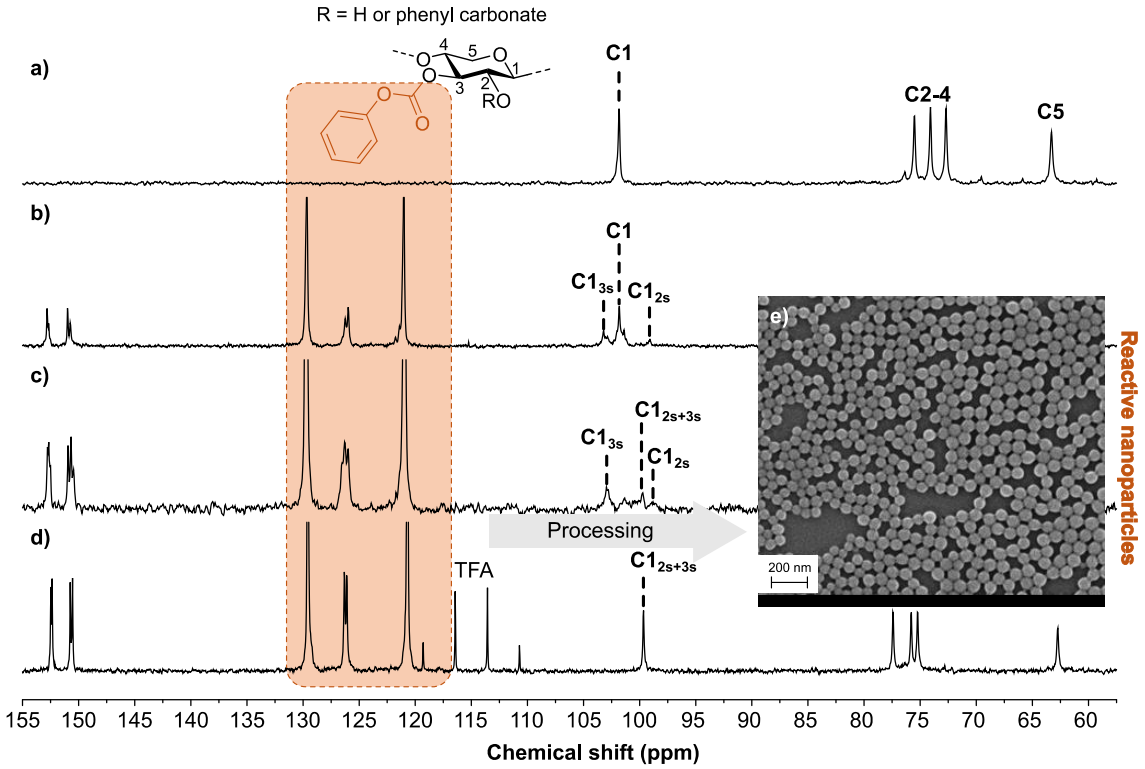

Fig. 3. ^{13}C NMR spectra (DMSO-d_6) of xylan (a) and xylan phenyl carbonate (XPhC) with rising degree of substitution of 0.46 (b), 1.17 (c), and 1.98 (d); scanning electron microscopy image of reactive nanoparticles from XPhC, DS = 1.54 (e); TFA = trifluoroacetic acid

CHEMICAL MODIFICATION OF CELLULOSE PHENYL CARBONATES

Advantages

Polysaccharide carbonates in general and polysaccharide phenyl carbonates in particular are promising reactive biopolymer derivatives for the design of advanced products and materials based on the most important renewable resource cellulose by conversions with nucleophilic compounds. Thus, anionic, cationic, and ampholytic polymers are accessible by advanced organic chemistry, as shown in Fig. 5.

Research revealed that the chemical modification of cellulose phenyl carbonates can be made with different nucleophiles in one-step reactions or stepwise. For nucleophiles with more than one reactive site with comparable reactivity, only one reactive moiety should be available; the other ones must be protected to exclude crosslinking and hence insoluble products. For amino-functions, typical protecting groups such as alkyloxycarbonyl moieties are well appropriate. Moreover, *p*-aminobezylamin yields organo-soluble *p*-aminobenzylcellulose carbamate (Ganske *et al.* 2016). Additionally, ethylene diamine moieties were introduced into the biopolymer *via* the protected intermediate as discussed above. The ω-aminoethylcellulose carbamate itself possesses a strong activity against *Candida albicans* and *Staphylococcus aureus* that is improved by *p*-aminobenzylamine substituent. Moreover, the mixed cellulose carbamate exhibits a high biocompatibility and forms films on cotton and PES that exhibit a strong activity against *Staphylococcus aureus* and *Klebsiella pneumonia*.

Even regioselective functionalization is possible, which is nicely documented by novel zwitterionic biopolymer derivatives of cellulose, *i.e.*, the positively charged ammonium groups present at position 6 while the negative charge of carboxylate is located at positions 2 and 3 of the repeating unit (Fig. 5). This uniform structure of the novel polymeric zwitterion could be realized by reaction of cellulose with 1.3 eq. phenyl chloroformate at 0 °C in DMAc/LiCl to induce selective modification of the primary OH at position 6. The subsequent aminolysis of the carbonate takes place at the activated moiety only, yielding 6-*O*-(*N*-Boc-2-aminoethyl) cellulose carbamate. Subsequently, phenyl chloroformate was allowed to react with the 6-*O*-(*N*-Boc-2-aminoethyl) cellulose carbamate; the product was treated with β-alanine ethyl ester to introduce the latent anionic moiety. Finally, deprotection leads to the zwitterionic product (Elschner *et al.* 2016). The antimicrobial activity of a coating based on this zwitterionic biopolymer derivative was demonstrated by confocal laser scanning microscopy and live/dead staining. As support material, glass, titanium, or tissue culture poly(styrene) were used. The antimicrobial activity was influenced by the support material. Additionally, for the polysaccharide dextran a regioselective functionalization is possible without protecting any hydroxyl group. Using the *p*-nitrophenyl carbonate of dextran, a regioselective substitution at C2 hydroxyl group with a DS of 1.0 is observed. Also for dextran a complete modification with maleimido group could be achieved starting from the dextran furfuryl carbamate. Processing the derivative into reactive films by spin-coating allowed the immobilization of protein (Fig. 4) (Elschner *et al.* 2017).

Unlike the polysaccharide tosylates, the subsequent modification of polysaccharide phenyl carbonate by aminolysis is not limited to a distinct group of polysaccharides. The reaction with functional amines is well transferrable. Further, not only the synthesis of cellulose phenyl carbonates works successfully for other polysaccharides than cellulose, but also the subsequent aminolysis is efficient (see section clickable polysaccharide derivatives).

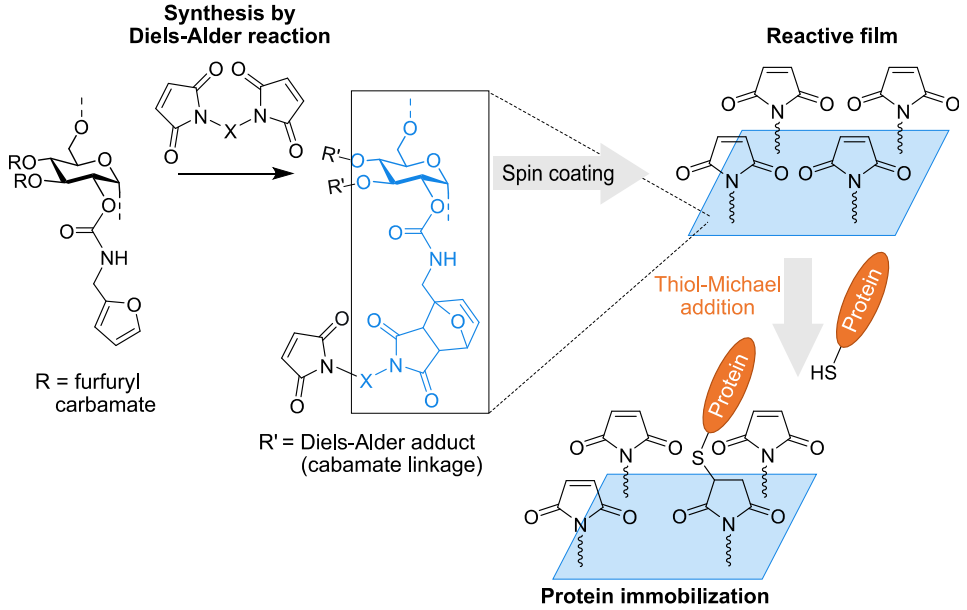

Fig. 4. Dextran furfuryl carbamate with a DS = 3.0, subsequent "click" reaction to obtain a maleimido-modified derivative (polysaccharide backbone and linker structure in blue), and processing of the maleimido derivative into reactive films for immobilization of proteins.

Limitations

Other than the undesired crosslinking of the carbonate during synthesis (see section synthesis of polysaccharide carbonates), there is another path of crosslinking of cellulose carbonate of low DS (0.3) by aminolysis. It became obvious that the primary carbonate is more reactive compared to the secondary one; even remaining hydroxyl groups may undergo S_N reaction and hence crosslinking. Nevertheless, there are strategies to overcome this undesired side effect. The crosslinking reaction can be avoided using protected 6-*O*-triphenylmethyl cellulose for the carbonate synthesis, on the one hand. On the other hand, the reactivity and stability of the cellulose carbonates could be controlled by substituents in the *p*-position of the phenyl ring, such as methoxy-, nitro-, or chloro group. Thus, cellulose phenyl carbonates with low DS of 0.3 were also accessible (Ganske and Heinze 2018). While the *p*-NO_2-substituent leads to regioselective substitution for dextran, and can be used successfully for other polysaccharides that only have secondary hydroxyl groups, such as xylan, it can only be used to a limited extent with a protective group for polysaccharides with a primary OH group. An example of the necessary use of the *p*-nitrophenyl carbonate of dextran is the synthesis of furfuryl modified dextran. Here the more reactive *p*-nitrophenyl carbonate reacted with furfuryl amine to form the corresponding carbamate (Elschner *et al.* 2017).

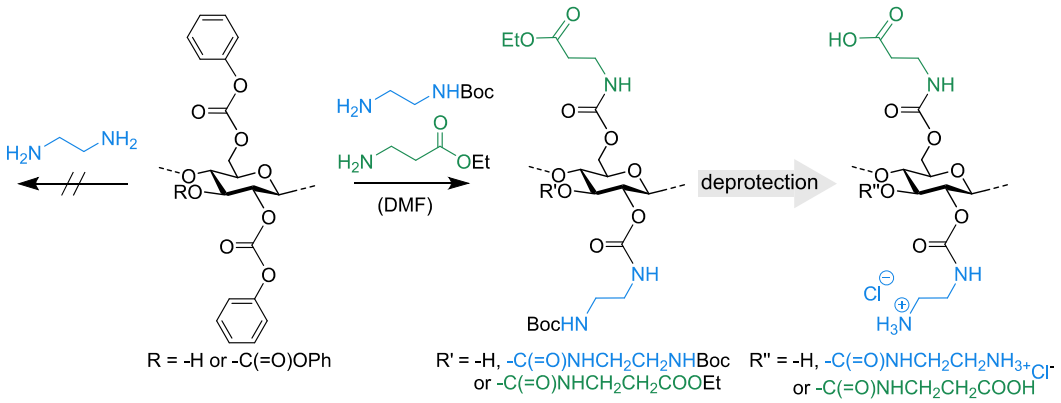

Fig. 5. Reaction scheme for the synthesis of cellulose carbamates with anionic, cationic, and ampholytic moieties (adapted from Elschner and Heinze 2014)

As mentioned, nucleophiles with two or more aliphatic amino moieties lead to crosslinking, while aromatic amino moieties do not possess sufficient nucleophilicity to be able to react with cellulose phenyl carbonate. Thus, amine protecting groups are necessary (Fig. 5) in contrast to the use of the polysaccharide tosylate derivatives, which can be reacted with unprotected diamines without crosslinking (Fig. 2).

Clickable Polysaccharide Derivatives Obtained from Tosylates and Carbonates

Further, the nucleophilic substitution reaction of polysaccharide tosylates and –phenyl carbonates can be used for the synthesis of derivatives that can be exploited for click chemistry approaches (Fig. 6). The use of clickable groups has a great influence on the sustainability of the process. The reactions mentioned, such as the Diels-Alder reaction with maleimide, the azide-alkyne reaction of the corresponding modified polysaccharide derivatives, and the polysaccharide allyl/ norbornene derivative for the thiol-ene reaction,

all have a high atom economy. The reactants (with the exception of the catalytic amounts of initiator) are completely incorporated into the product. The reactions can also be carried out in aqueous media under mild conditions and with appropriate previous functionalization of the polysaccharides.

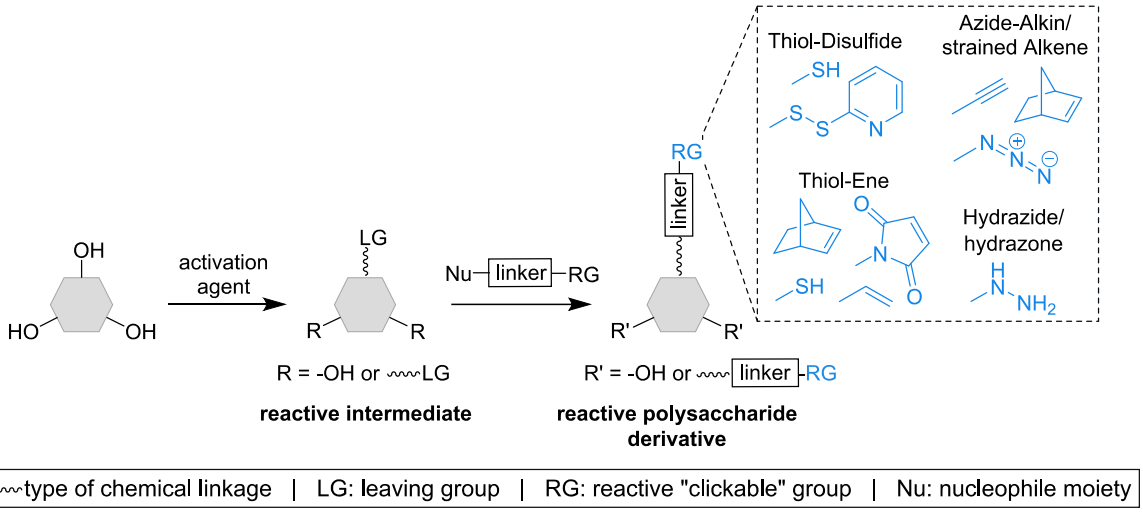

Fig. 6. General scheme for cellulose tosylate and phenyl carbonate as reactive intermediates towards reactive "clickable" derivatives

An important type of click chemistry is the Huisgen reaction azide–alkyne cycloaddition. The reaction can be exploited for the preparation of various cellulose derivatives (Abdellatif *et al.* 2015). Because of the catalysis by a Cu(I) species (commonly copper(II) sulfate and sodium ascorbate) the conversion can be completed efficiently under mild conditions and even in aqueous media. The two reactive groups needed for the Huisgen reaction are an azide- and an alkyne moiety that can be easily introduced by S_N reaction with cellulose tosylate. The azide anion is one of the strongest nucleophiles and reacts well with cellulose tosylate to regioselectively yield 6-deoxy-6-azide cellulose, *i.e.*, the primary tosylates are substituted only (Heinze *et al.* 2006b). The alkyne containing cellulose derivative may be obtained from cellulose tosylate by S_N reaction with propargylamine (Koschella *et al.* 2011). Both polymers may react with each other yielding hydrogels. The activated cellulose derivatives can be functionalized to a myriad of novel biopolymers with organic compounds containing azide- and alkyne moieties. Dendron-like moieties could be attached to cellulose (Montanez *et al.* 2011). Either the azide (Heinze *et al.* 2008) or the alkyne moiety was attached to the complementarily modified polymer backbone (Pohl and Heinze 2008).

Oligosaccharides such as *O*-linked β-maltoside and β-lactoside containing terminal alkyne could be linked to 6-azido-6-deoxycellulose (Negishi *et al.* 2011). This approach was successfully applied for the preparation of novel all sugar-based cellulose derivatives (Fig. 7). The clickable groups attached to the cellulose (azide- or alkyne moiety) were allowed to react with modified sugar compounds containing the corresponding reactive group. With the modified sugars, up to 100% of the reactive moieties on the polymer backbone could be converted, whereby the conversion rate is dependent on the steric demand of the components (Koschella *et al.* 2020b).

One of the first papers to discuss clickable chemistry with cellulose was published in 2006 (Liebert *et al.* 2006). 6-Azido-6-deoxy cellulose obtained by S_N reaction of

cellulose tosylate with sodium azide was allowed to react with methyl propiolate, 2-ethynylaniline, and 3-ethynylthiophene, yielding the corresponding cellulose derivatives. The substituents are attached *via* the 1,4-disubstituted 1,2,3-triazol linker. The 1,3-dipolar cycloaddition catalyzed with copper(I) (copper(II) sulfate pentahydrate/sodium ascorbate) occurs regioselectively regarding the 1,2,3-triazole and under mild reaction conditions. Moreover, the distribution of the substituents is also limited to the primary hydroxyl group at the C6-atom because of the regioselective introduction of the azide moieties.

Not only organo-soluble 6-azido-6-deoxy cellulose but also water-soluble derivatives are known. The biopolymer derivative can be transferred to a water-soluble one by modification of the remaining OH group at the secondary positons 2 and 3 of the AGU with ionic moieties. A cationic moiety 4-(*N*,*N*,*N*-trimethylammonium)butyrate chloride can be attached to the 6-azido-6-deoxy cellulose to result a water-soluble product (DS of cationic group up to 0.24). Alternatively, etherification with 4-bromobutyltrimethyl-ammonium bromide yielded 6-azido-6-deoxy-2,3-*O*-(4-trimethylammonium)butyl cellulose bromide with a similar DS. These polymers are reactive and can be modified *via* azide–alkyne click chemistry in aqueous media. These polymers are reactive and can be modified *via* azide–alkyne click chemistry (Bretschneider *et al.* 2015).

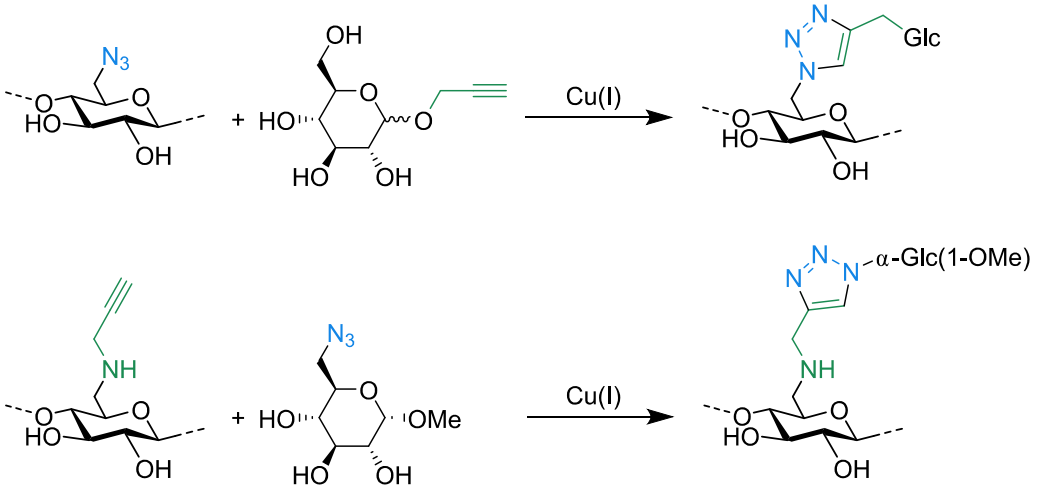

Fig. 7. Reaction scheme for the preparation of sugar modified cellulose derivatives by Huisgen click reaction (Cu(I) catalyzed azide–alkyne 1,3-dipolar cycloaddition); Me = methyl

The simple heterogeneous carboxymethylation of 6-azido-6-deoxy cellulose applying 2-propanol/aqueous NaOH as slurry medium represents another path to obtain water-soluble precursors for the azide–alkyne click chemistry (Pohl *et al.* 2009). It is important to note that under the drastic alkaline conditions needed for the etherification, the azido moieties are stable. Chemoselective dendronization could be achieved by copper-catalyzed azide-alkyne reaction, yielding water-soluble carboxymethyl-6-deoxy-(1-*N*-[1,2,3-triazolo]-4-polyamidoamine) cellulose (first generation with DS = 0.51, second generation with DS = 0.44, and third generation with DS = 0.39). The dendronized cellulose possesses a persistence length of 2.8 to 4.0 nm without any change in flexibility, as could be concluded from conformation zoning. A comparable approach was used to get the water-soluble carboxymethyl-6-deoxy-6-azdio cellulose for crosslinking with 6-deoxy-6-aminopropargyl cellulose in water (Koschella *et al.* 2011).

Another approach, which deals with the preparation of a clickable derivative using nucleophilic substitution on the polysaccharide, was reported for the synthesis of dextran furfuryl carbamate. After synthesis of the derivative, the Diels-Alder reaction was completed successfully with a bismaleimide linker to obtain reactive films (Fig. 4). These films could be applied for the surface immobilization of the protein bovine serum albumin (Elschner *et al.* 2017).

To make thiol-ene reaction better accessible for highly modified polysaccharides, the activated starting material cellulose phenyl carbonates with DS from 2 to 3 were allowed to react with allylamine resulting in the clickable cellulose allyl carbamate with high DS of up to 2.2 (Fig. 8). Pure products without remaining phenyl carbonate substituents were accessible, but also mixed products with remaining phenyl carbonate moieties (Lindemann and Heinze 2022). The phenyl carbonate groups can be used for modification with further functional moieties, for example to adjust the physico-chemical properties. In addition to other propene-containing cellulose derivatives in the form of allyl ether or acrylate units, this path is an important alternative that despite the two-step synthesis, is highly efficient because a higher content of reactive C=C bonds attached to the polymer backbone is achieved. The high reactivity of these C=C double bonds with thiols could be demonstrated in a model reaction with the cationic thiol *N*,*N*-diethylamine ethanethiol hydrochloride. This makes the cellulose allyl carbamate into an appealing carrier polymer. A comparable chemistry can be carried out with propargylamine and a functional amine with terminal azido moiety as well (Fig. 8). The polysaccharide phenyl carbonate reacts readily with propargylamine under formation of the corresponding carbamate containing the reactive triple bond. In a similar way, the corresponding azide functionality could be attached to the polysaccharide (Skodda 2022).

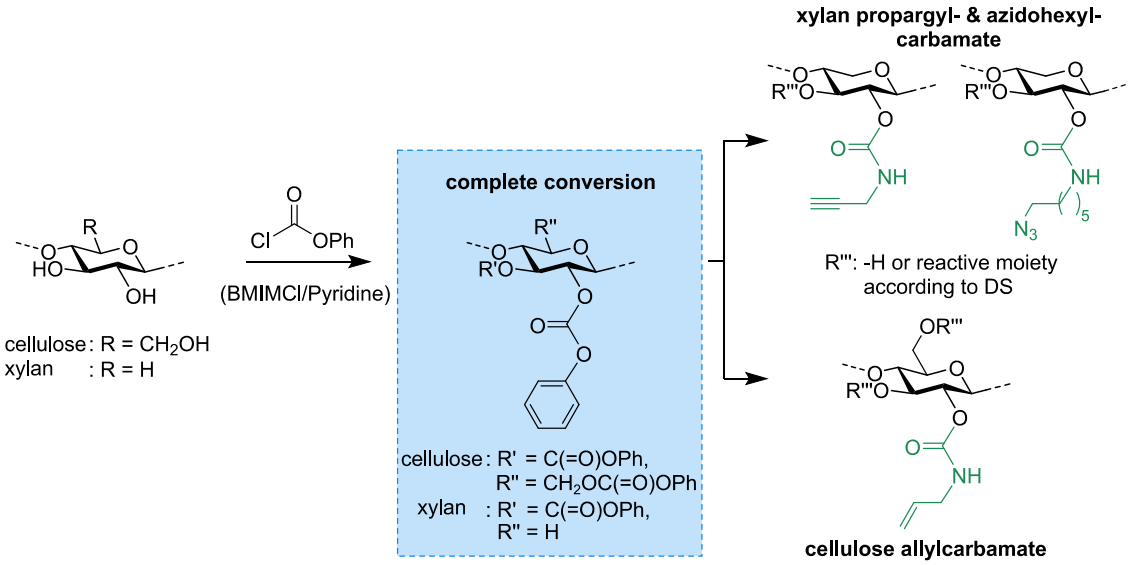

Fig. 8. Reaction scheme for the synthesis of clickable cellulose/xylan carbamates by the conversion of cellulose phenyl carbonate with amines; BMIM Cl = 1-butyl-3-methylimidazolium chloride

CONCLUSIONS

It became obvious that the synthesis paths discussed by conversion of the polysaccharide to the polysaccharide tosylate or polysaccharide phenyl carbonate as reactive intermediates and the functionalization by nucleophilic substitution is a promising approach useful for chemical modification of polysaccharides that contain hydroxyl groups, such as neutral polysaccharides. The advantages of this chemistry clearly outweigh the limitations. It is obvious that the structural diversity of the chemically modified derivatives is enhanced. To make optimal use of the advantages of the nucleophilic substitution of the respective derivative, recommendations for the synthesis can be given. For the synthesis of non-natural polysaccharides with amino groups, the reactive polysaccharide tosylate provides the starting compound of choice, even though compounds with terminal amino groups also could be produced using polysaccharide carbonates. In contrast, polysaccharide phenyl carbonates can be used for screening the optimal composition of the polymer, as in a modular synthesis approach different amines can be used in a one-pot reaction, *e.g.*, using a functional group and a solubility-mediating group. Functional polymers with different properties can be obtained by varying the molar ratio of both substituents alone. It may be expected that nucleophilic displacement reaction with polysaccharide will be increasingly applied for the design of advanced polysaccharide derivatives.

Although the synthesis method presented here is convincing, it is not suitable for optimizing already established technical processes. The multi-step synthesis with the reactive intermediate tosylate or phenyl carbonate formally adds a step, but the efficiencies are very high, and the conversion rates on average are higher than with a direct modification of the polysaccharide. The reactive intermediates can be used primarily to bind various reactive groups to the polymer backbone in a targeted and selective manner. This can be particularly advantageous for applications in the biomedical/ pharmaceutical field, where different functionalities (drug, dye, targeting group, solubility mediator, crosslinker) need to be introduced. The modular approach is also advantageous in this field, as many nucleophiles are suitable for the incorporation of the clickable groups, which can be easily adapted. The click reaction can be carried out with a variety of structures for the final functional moiety. The combination possibilities can contribute to creating a polymer library from the same reactive starting material. This step of the process can be easily adapted without the need for extensive optimization of reaction conditions.

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