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New 6-butylamino-6-deoxycellulose and 6-deoxy-6-pyridiniumcellulose derivatives with highest regioselectivity and completeness of reaction

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Abstract—This paper investigates the nucleophilic substitution (S_N) reactions of tosylcellulose with butylamine and pyridine, respectively. The S_N reactions of tosylcellulose **1** (DS_{Total} 2.02; $DS_{C\text{-}6}$ 1.0) with butylamine carried out at 25, 50, 75 and 100 °C in both dimethyl sulfoxide (DMSO) and pure butylamine showed that the regioselectivity of substitution at C-6 of cellulose is temperature dependent: the highest regioselectivity at C-6 can be reached at 25 and 50 °C; substitution at C-2 also occurred at 75 and 100 °C. The substitution speed in pure butylamine is greater than that in the presence of DMSO. A complete and regioselective substitution at C-6 with a DS of 1.0 was obtained under the conditions of 50 °C, 40 h in butylamine. The substitution reactions of **1** with pyridine carried out at 25, 50, 75 and 100 °C for 24 h in DMSO did not occur. In contrast to this the S_N reactions done in pure pyridine showed that a temperature- and steric-dependent, regioselective substitution took place at C-6 at temperatures from 25 to 145 °C. The highest regioselectivity and completeness at C-6 can be obtained at 100 °C for 90 h, whereas at 145 °C substitution also occurs at C-2. The results were proved by ^1H NMR and ^{13}C NMR spectroscopy.

Keywords: Regioselective modification; Temperature dependence; Nucleophilic substitution; Steric effect; Tosylcellulose

1. Introduction

Completely and partially regioselectively modified polysaccharides with different backbones and different functional groups (OSO_3^-, NSO_3^-, NAc, COO^-, etc.) at different positions of the repeating units are important substances in living organisms for influencing a variety of normal and pathological processes, such as tumour growth, metastasis, inflammation, autoimmunity, angiogenesis, morphogenesis, cell proliferation, platelet adhesion and blood coagulation.[1–5]

For more than 20 years, there has been a growing interest in the synthesis of polysaccharide mimetics with a variety of functional groups.[6–8] Classical stepwise syntheses,[9] protecting group strategy,[10] enzymatic synthesis[11] and solid-phase synthesis,[12] as well as ring-opening polymerization,[13] are limited for preparing regioselectively modified polysaccharide mimetics with high molecular weight and different functional groups. However, regioselective polymer analogue modification of polysaccharides may solve these problems.[14] One of the most important applications of selective modifications is the synthesis of substituted polysaccharides, such as chitosan,[15–17] heparin,[18] cellulose[19–22] or cyclodextrin.[23,24]

Cellulose is most abundant among renewable natural polymers and has three hydroxyl groups, one anomeric center and four asymmetric carbon atoms in every anhydroglucose repeating unit. Therefore, the potential to prepare different functionalized polymers is of great interest for industrial and medical applications. The trityl group is a specific protecting group for the C-6

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position and has often been used to prepare a number of partially substituted 6-deoxycellulose derivatives.[25–29] This is also the case with less specific reaction of the tosyl group at the C-6 position. The tosyl group, which is a good leaving and activating group, reacts directly with various nucleophiles. Water-soluble 6-deoxy-6-trialkylammonium cellulose derivatives with a DS range from 0.2 to 0.5 were obtained.[30] Heinze et al.[31] reported on the 6-deoxy-6-aminocellulose derivatives with a DS from 0.4 to 0.6 via tosylcellulose with chiral and racemic amines. Deoxyiodocelluloses with a DS from 0.46 to 0.94 have been synthesized by iodination of tosylcellulose.[32] Tiller et al.[33] prepared 6-deoxy-6-(4-aminophenyl)amino-2(3)-*O*-tosylcellulose with a DS from 0.28 to 0.86. Among these literature reports, only Rahn et al.[32] observed that displacement of tosylcellulose with sodium iodide was not specific at C-6, but also occurred at C-2 at high temperatures. However, the authors did not improve the regioselectivity and completeness of the reaction.

Only a few well-characterized derivatives are completely substituted at the C-6[20,21] or C-3[22] positions of cellulose. There are also few completely substituted derivatives with the same functional groups in two positions (C-6 and C-2).[19,34]

In a recent paper,[20] we reported that the highest regioselective and complete substitution occurred at the C-6 position of cellulose with the help of a temperature-dependent nucleophilic substitution of fully C-6 tosylated cellulose with NaN_3 and NaI, respectively. In this paper, we also use the temperature-dependent nucleophilic substitution of tosylcellulose with butylamine and pyridine to synthesize new 6-amino-6-deoxycellulose derivatives with the highest regioselectivity and completeness without using protecting groups.

2. Experimental

2.1. Starting materials

Cellulose was purchased from Fluka Chemical Co. (AVICEL PH-101) with DP 280. Other chemicals and solvents were purchased either from Fluka or Aldrich Chemical Co. All chemicals and reagents, unless otherwise specified, were not further purified, dried or pretreated. *N*,*N*-Dimethylacetamide (DMAc) was dried over calcium hydride and subsequently distilled in vacuum.

2.2. Analytical methods

^{1}H NMR and ^{13}C NMR spectra of cellulose derivatives were measured with a Bruker AC 300 spectrometer operating at 300 MHz and 75.47 MHz in Me_2SO-d_6, respectively. The degree of substitution was calculated by the 'triangle method' according to Casu et al.[35] The infrared spectra were measured in KBr pellets with an IMPACT 400 FTIR spectrometer from Nicolet.

2.3. *p*-Toluenesulfonylation of cellulose 1

Cellulose (5.0 g, 30.8 mmol) was suspended in DMAc (120 mL) and stirred at 160 °C for 1 h. In order to remove the water bound to cellulose, about 12 mL of DMAc were removed by distillation under a N_2 atmosphere. After the mixture cooled to 100 °C, 10.0 g of anhyd LiCl (dried at 130 °C in vacuum overnight) was added. By cooling down to RT and continuous stirring, the cellulose was dissolved completely within several hours, resulting in a bright-yellow viscous solution.

A mixture of triethylamine (38.5 mL, 276.0 mmol) in DMAc (25 mL) was added to the solution of cellulose under stirring. After cooling to 8 °C, a solution of *p*-toluenesulfonyl chloride (26 g, 136.9 mmol) in DMAc (56 mL) was added dropwise within 1 h. The homogeneous reaction mixture was stirred for 24 h at 8 °C under an N_2 atmosphere and then slowly poured into ice-cold water (1.2 L). The precipitate was filtered off, washed carefully with about 5 L of distilled water and 600 mL of EtOH, dissolved in 250 mL of acetone and reprecipitated in ≈800 mL of distilled water. After filtration and washing with EtOH, the product was dried at 50 °C in vacuum. (**1**) Yield: 82%. Total DS of tosyl group: 2.02 (by elemental analysis); $DS_{C\text{-}6}$: 1.0 (by ^{13}C NMR); $DS_{C\text{-}2}$: 0.85 (by ^{13}C NMR); $DS_{C\text{-}3}$: 0.17 (by elemental analysis and ^{13}C NMR). IR (KBr): ν 3515 (OH), 3075 (C-H_{arom}), 2924 (CH), 1600 and 1458 (C–C_{arom}), 1370 and 1180 cm^{-1} (SO_2). ^{13}C NMR (Me_2SO-d_6): δ 144.9–127.7 (C-H_{arom}), 101.5 (C-1), 97.9 (C-1′), 83.3–71.6 (C-2, C-2-O-Ts, C-3, C-3-O-Ts, C-4, C-5), 68.2 (C-6-O-Ts), 21.1 (CH_3) ppm.

2.4. S_N reaction of tosylcellulose with butylamine in DMSO

In a typical reaction, 6-*O*-tosylcellulose (**1**, 400 mg, 0.85 mmol) was dissolved in DMSO (25 mL). To this solution, butylamine (310 mg, 4.3 mmol) was added. After stirring for 24 h at 75 °C under an N_2 atmosphere, the solution was poured slowly into 2-PrOH (40 mL), filtered off, washed with 2-PrOH and dried in vacuum at 50 °C overnight. (**2c**) Yield: 80%. ^{1}H NMR (Me_2SO-d_6): δ 7.7–7.4 (C_6H_4-Me), 5.5–3.0 (C*H*-Cellulose unit), 2.5–2.2 (CH_2, Ph-CH_3), 1.3–0.8 (CH_2-CH_2CH_3), ppm; ^{13}C NMR (Me_2SO-d_6): δ 144.9–127.6 (C-H_{arom}), 103.9 (C-1″), 100.8 (C-1), 97.1 (C-1′), 84.0–68.7 (C-2, C-2-O-Ts, C-3, C-3-O-Ts, C-4, C-5, C-6-O-Ts), 55.8 (C-2-NHBu), 48.8 (C-6-NHBu), 31.5 ($NHCH_2C_3H_7$), 29.9 ($NHCH_2CH_2C_2H_5$), 21.1

(Ph*C*H$_3$), 19.8 (NHC$_2$H$_4$*C*H$_2$CH$_3$), 13.8 (NHC$_3$H$_6$*C*H$_3$) ppm.

2.5. S_N reaction of tosylcellulose with butylamine without additional solvent

In a typical reaction, 6-*O*-tosylcellulose (**1**, 400 mg, 0.85 mmol) was dissolved in butylamine (8 mL). After stirring for 24 h at 50 °C under an N_2 atmosphere, the solution was poured slowly into 2-PrOH (40 mL), filtered off, washed with 2-PrOH and dried in vacuum at 50 °C overnight. (**2f**) Yield: 82%. ^{1}H NMR (Me$_2$SO-d_6): δ 7.4–7.1 (C$_6$*H*$_4$-Me), 5.5–3.0 (C*H*-Cellulose unit), 2.5–2.2 (C*H*$_2$, Ph-C*H*$_3$), 1.3–0.8 (C*H*$_2$C*H*$_2$C*H*$_3$), ppm; ^{13}C NMR (Me$_2$SO-d_6): δ 144.7–128.1 (C-H$_{arom}$), 102.2 (C-1), 98.3 (C-1′), 79.6–70.4 (C-2, C-2-O-Ts, C-3, C-3-O-Ts, C-4, C-5), 48.8 (C-6-NHBu), 31.7 (NH*C*H$_2$-C$_3$H$_7$), 30.2 (NHCH$_2$*C*H$_2$C$_2$H$_5$), 21.1 (Ph*C*H$_3$), 19.9 (NHC$_2$H$_4$*C*H$_2$CH$_3$), 13.9 (NHC$_3$H$_6$*C*H$_3$) ppm.

2.6. S_N reaction of tosylcellulose with pyridine in DMSO

In a typical reaction, 6-*O*-tosylcellulose (**1**, 400 mg, 0.85 mmol) was dissolved in DMSO (25 mL). Pyridine (0.35 mL, 4.3 mmol) was added to the solution. After stirring for 24 h at 100 °C under an N_2 atmosphere, the solution was poured into 2-PrOH (40 mL), filtered off, washed with 2-PrOH and dried in vacuum at 50 °C overnight. (**3d**) Yield: 84%. ^{1}H NMR (Me$_2$SO-d_6): δ 7.7–7.4 (C$_6$*H*$_4$-Me), 5.5–3.0 (C*H*-Cellulose ring), 2.5–2.3 (Cellulose-O*H*, Ph-C*H*$_3$), ppm.

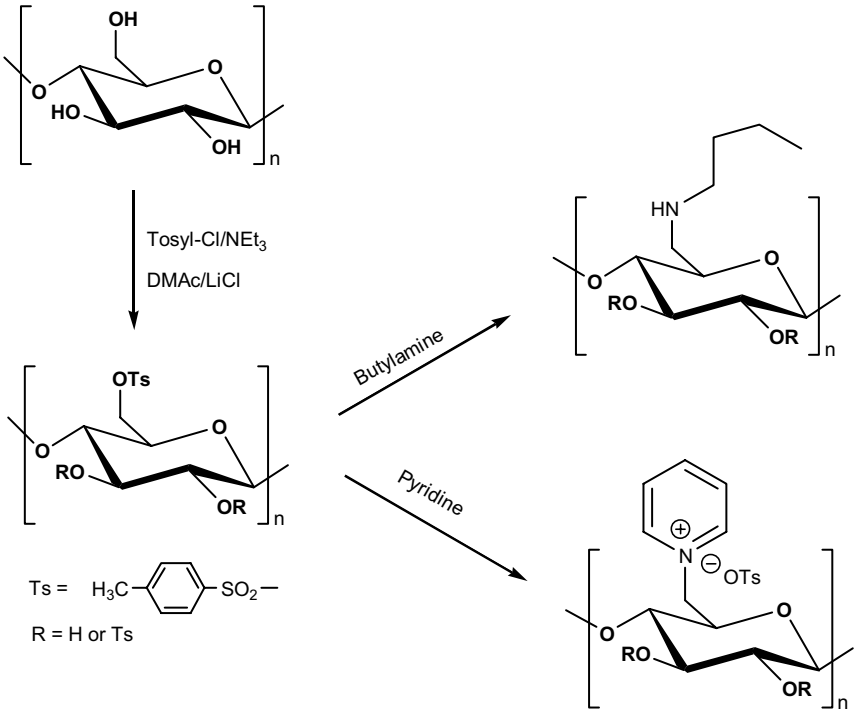
2.7. S_N reaction of tosylcellulose with pyridine without additional solvent

In a typical reaction, tosylcellulose **1** (400 mg, 0.85 mmol) was dissolved in dry pyridine (8 mL). After stirring for 3 days at 100 °C under an N_2 atmosphere, the solution was poured into 2-PrOH (40 mL), filtered off, washed with 2-PrOH and dried in vacuum at 50 °C overnight. (**3h**) Yield: 86%. ^{1}H NMR (Me$_2$SO-d_6): δ 8.7–7.9 (C$_5$*H*$_5$N), 7.5–7.0 (C$_6$*H*$_4$-Me), 5.5–3.0 (C*H*-Cellulose ring), 2.4–2.1 (C*H*$_2$, Cellulose-O*H*, Ph-C*H*$_3$), ppm; ^{13}C NMR (Me$_2$SO-d_6): δ 145.0–125.5 (C-H$_{arom}$), 102.1 (C-1), 98.2 (C-1′), 80.7–68.7 (C-2, C-2-O-Ts, C-3, C-3-O-Ts, C-4, C-5), 60.6 (C-6-Py), 20.8 (CH$_3$) ppm.

3. Results

3.1. General approach

6-*O*-Tosylcellulose is a very useful intermediate in cellulose chemistry.[36] The tosyl group can serve as both a leaving group and an activating group in chemical modification of cellulose derivatives. Moreover, cellulose derivatives containing the tosyl moiety make it possible for the modification to be carried out in a homogeneous manner in common organic solvents. In this paper, we use a complete C-6 tosylated cellulose derivative **1** to study the nucleophilic substitution with butylamine and pyridine, respectively. Scheme 1 shows the regioselective



Scheme 1. Nucleophilic substitution of 6-*O*-tosylcellulose with butylamine and pyridine.

nucleophilic substitution of tosylcellulose against butylamine and pyridine, respectively. In order to obtain the highest possible regioselectivity at C-6 of cellulose, a fully 6-*O*-tosylated cellulose **1** was prepared in DMAc/LiCl.

3.2. S_N reaction of tosylcellulose with butylamine in DMSO

S_N reaction of 6-*O*-tosylcellulose (**1**) with butylamine was carried out in a mixture of DMSO and pure butylamine. The ^{1}H NMR spectra indicate that temperature has a great effect on the reaction speed. The degree of substitution of butylamino group (the area of 0.8–1.3 ppm) increased with the increasing of temperature. Table 1 shows the quantitative DS value of the substituted groups calculated according to the triangle method of Casu and co-workers[37] based on the ^{13}C NMR spectra.

At 75 and 100 °C the S_N reaction occurred at C-6 as well as at C-2, which can be seen in the ^{13}C NMR spectra. Figure 1 shows the ^{13}C NMR spectrum of butylaminodeoxy-cellulose tosylate **2d**. The signals of the carbons of the tosyl methyl and the aromatic ring appear at 21.1 ppm and 127.6–144.9 ppm, respectively. The signal of the C-6-butylamino is found at 48.8 ppm, which yields a strong highfield shift (C-6-OTs appears at 68.2 ppm). In the region of C-1, three peaks can be clearly distinguished, which means that there have to be C-2 atoms in three different states of substitution. The signals may be assigned as follows: 97.3 ppm C-1′ (C-2-OTs), 100.8 ppm C-1 (C-2-OH) and 102.6 ppm C-1″ (C-2-butylamino) . The ^{13}C NMR spectrum of **2b** obtained at 50 °C gives five new peaks compared with that of **1**. The signal at 48.8 ppm is assigned to the C-6-butylamino group. The other four signals at 31.5, 29.9, 19.8 and 13.8 are assigned to the carbon atoms of the butyl group. The percentage of tosyl groups displaced by butylamino groups at C-6 increased from 26% to 75%, while the reaction temperature was increased from 25 to 100 °C. At 75 °C, tosyl groups at C-2 with a DS of 0.05 were substituted by butylamino groups, while the DS of C-2-butylamino reached to 0.07 at 100 °C.

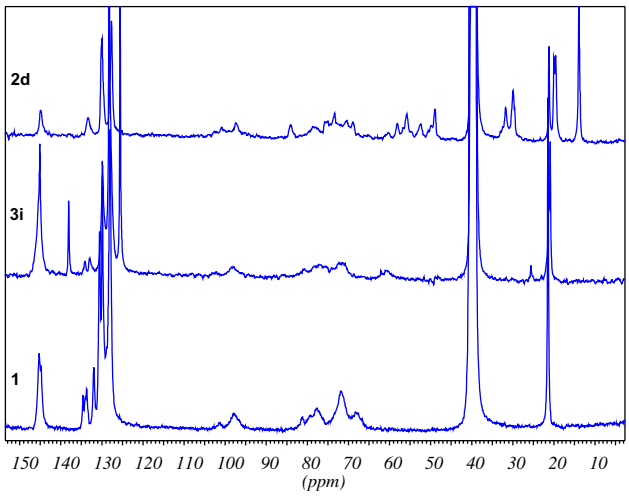


Figure 1. ^{13}C NMR spectra of tosylcellulose (**1**) and modified cellulose derivatives **2d** and **3i**.

3.3. S_N reaction of tosylcellulose with butylamine without using additional solvent

Derivatives **2e–g** were prepared by the S_N reaction of 6-*O*-tosylcellulose with butylamine without using additional solvent carried out at 25, 50 and 75 °C, respectively. The results are shown in Table 2. Compared with the results obtained in DMSO (**2a–c**), the reaction with pure butylamine is very fast at the same temperature. In the case of 25 °C, the amount of tosyl groups replaced by butylamino groups increased from 26% (**2a**) to 88% (**2e**). No substitution occurred at C-2 at the temperatures of 25 and 50 °C, while tosyl groups at C-2 with a DS of 0.08 were replaced by butylamino groups at 75 °C. The highest regioselectivity and completeness (100%) at C-6 was reached at 50 °C for 40 h (**2g**).

3.4. S_N reaction of tosylcellulose with pyridine in DMSO

The S_N reaction of 6-*O*-tosylcellulose (**1**) with pyridine was carried out at first at 25, 50, 75 and 100 °C for

Table 1. Conditions and results of the nucleophilic reaction of 6-*O*-tosylcellulose with butylamine

Entry[a]	Ba[b]/mL	Solvent	Temp/°C	Time/h	Partial DS of products calculated from ^{13}C NMR				
					$C_{2\text{-OH}}$	$C_{2\text{-Ts}}$	$C_{2\text{-Ba}}$	$C_{6\text{-Ts}}$	$C_{6\text{-Ba}}$
2a	0.42	DMSO	25	24	0.16	0.84	0.00	0.74	0.26
2b	0.42	DMSO	50	24	0.17	0.83	0.00	0.60	0.40
2c	0.42	DMSO	75	24	0.12	0.82	0.05	0.30	0.70
2d	0.42	DMSO	100	24	0.09	0.84	0.07	0.25	0.75
2e	8.0		25	24	0.18	0.82	0.00	0.12	0.88
2f	8.0		50	24	0.16	0.84	0.00	0.08	0.92
2g	8.0		50	40	0.15	0.85	0.00	0.00	1.00
2h	8.0		75	24	0.10	0.82	0.08	0.10	0.90

[a] In all cases, the amount of 6-*O*-tosylcellulose was 400 mg.
[b] Ba: butylamine.

Table 2. Conditions and results of the nucleophilic reaction of 6-*O*-tosylcellulose with pyridine

Entry[a]	Py[b]/mL	Temp/°C	Time/h	Partial DS of products calculated from ^{13}C NMR				
				$C_{2\text{-OH}}$	$C_{2\text{-Ts}}$	$C_{2\text{-Py}}$	$C_{6\text{-Ts}}$	$C_{6\text{-Py}}$
3e	8.0	25	72	0.15	0.85	0.00	1.00	0.00
3f	8.0	50	72	0.14	0.86	0.00	0.85	0.15
3g	8.0	75	72	0.14	0.86	0.00	0.16	0.84
3h	8.0	100	72	0.15	0.85	0.00	0.04	0.96
3i	8.0	100	90	0.16	0.84	0.00	0.00	1.00
3j	8.0	145	48	0.06	0.86	0.08	0.00	1.00

[a] In all cases, the amount of 6-*O*-tosylcellulose was 400 mg.
[b] Py: pyridine.

24 h in DMSO (**3a–d**). However, no substitution occurred according to the ^{1}H NMR spectra (not shown).

3.5. S_N reaction of tosylcellulose with pyridine without using additional solvent

Cellulose derivatives **3e–h** were synthesized by the S_N reaction of 6-*O*-tosylcellulose (**1**) with pyridine in pure pyridine for 3 days at 25, 50, 75 and 100 °C, respectively. The partial DS of functional groups was calculated according to the triangular ^{13}C NMR method and is shown in Table 2. At 25 °C, no displacement was observed. This was proved by the ^{13}C NMR and ^{1}H NMR spectra of **3e**, **3e** and **1**, all of which gave the same spectrum. Compared with the ^{13}C NMR spectrum of **1**, a new chemical shift at 60.6 ppm is observed in those of **3f–i**, which can be assigned to the C-6-pyridinium moiety (Fig. 1). In contrast to this at 145 °C (**3j**), an additional new signal at 49.2 ppm for C-2-pyridinium is observed. At temperatures of no more than 100 °C, the highest regioselectivity can be obtained, and the amount of selective substitution at C-6 is dependent on the temperature. The content of tosyl groups at C-6 of **1** substituted by pyridine was reached from 15% to 96% when the temperature was increased from 50 to 100 °C for 72 h. At longer reaction time the highest regioselectivity and completeness (100%) was obtained at 100 °C for 90 h (**3i**).

4. Discussion

This selected temperature-dependent pathway to introduce specific functional butylamino and pyridyl residues into the C-6 position of cellulose is elegant in that it can attain the highest regioselectivity and completeness in only a few reaction steps. Ostensibly, this seems to be bold chemistry because we make use of simple, known literature procedures that are often cited for the tosylation of OH groups[20,32,36] and nucleophilic substitution reactions[25,28,31,33] to realize the above-mentioned high standards. Although a larger number of reaction steps were needed with nearly all the other approaches,[9–13] they were either not successful or did not reach such a comparable high standard with relative high molecular weight cellulose derivatives as do the present procedures.

Cellulose derivatives with the highest regioselectivity and completeness with the same group in one position (C-6 or C-3) or in two positions (C-6 and C-2) have been reported only a few times.[19–22,34] Only three fully C-6 substituted cellulose derivatives, 6-amino-6-deoxycellulose, 6-azido-6-deoxycellulose and 6-deoxy-6-iodocellulose derivatives,[20] have been characterized completely in terms of identity and purity using the known standard 'triangle method' developed by Casu et al.,[35,37] developed nearly two decades ago based on ^{13}C NMR spectroscopy. The other reported fully substituted derivatives, 2,6-di-*O*-sulfocellulose,[34] 3-*O*-allylcellulose[22] and 3-*O*-methylcellulose[22] derivatives, have so far only been quantitatively characterized by elemental analysis and additionally by their qualitative ^{13}C NMR spectra. The ^{13}C NMR method was also successfully used for quantitating partially substituted chitosan derivatives[15,16] and even more complex substituted polysaccharides such as heparin.[18,37–39] Six different functionalized heparin groups were quantified with one ^{13}C NMR spectrum.[37] The prerequisite is a minimum of 20,000 scans and a 300 MHz NMR spectrometer to get high resolution. This means that the quantitative ^{13}C NMR method is sufficient for characterizing our two target products, which are fully and exclusively substituted in the C-6 position. We did not find any overlapping of peaks of C-6 carbon atoms with that of any other carbon atom in the cellulose repeating units. This is also true for C-6-amino,[20] C-6-azido[20] and C-6-iodo[20,32] cellulose derivatives.

It is clear that the prerequisite for preparing the regioselecitvely modified target products is a complete tosylation at the C-6 position of cellulose, which has only a few times been realized in literature reports.[20] Because the steric effect of the tosyl group is not large enough, in comparison to a trityl group,[25,28,29] we must accept partial tosylation in the C-2 and C-3 positions when the C-6 position is fully tosylated.[20] However, the tosyl groups in the C-2 and C-3 positions can be removed completely by reduction at the end of the pathway.[20]

The attack of tosylated OH groups in the C-6, C-2 and C-3 positions of cellulose by nucleophiles will be influenced by the reactivity of the nucleophilic amines and the steric effects of their alkyl or aromatic residues, the different steric effects of the anhydroglucose units in the C-2 and C-3 positions, and the steric effect of the tosyl group, in contrast with the C-6 position, which is easy available. These effects will be influenced strongly by reaction temperature. Lower temperatures prefer specific nucleophilic substituted products at the C-6 position of cellulose, whereas higher temperatures result in partially substituted products at C-6, as well as at C-2 and/or C-3 positions.

The specific and typical low reaction temperature range for each nucleophile is as follows: sodium azide (25–50 °C), sodium iodide (25–60 °C), butylamine (25–50 °C) and pyridine (25–100 °C). This shows, among others, the importance of the steric effects of nucleophiles on nucleophilic substitution.

At a constant temperature of 100 °C there are different reaction ratios in partially substituted cellulose derivatives: C-6-azido, C-2-azido and C-3-azido, 86:12:5;[20] C-6-iodo and C-2-iodo, 84:7;[20] C-6-butylamino and C-2-butylamino, 75:7 (**2d**); C-6-pyridyl and C-2-pyridyl is 90:0 (**3h**).

The content of the C-3-iodo, C-3-butylamino and C-3-pyridinium derivatives cannot be determined by ^{13}C NMR spectroscopy because their peaks are overlapping with others, but it was possible to calculate the content of each in combination with additional elemental analyses.[32] However, these partially substituted derivatives are not our target products. The results show that the aromatic pyridyl residue with the stronger steric effect reaches the highest degree of regioselectivity and completeness, whereas that of the alkyl butylamino group at the same temperature (100 °C) leads to only partial substitution at C-6, C-2 and C-3.

^{1}H NMR spectroscopy is not sufficient to characterize quantitatively all of the above-mentioned derivatives, because the chemical shifts of the protons in the C-2 and C-3 positions are overlapping with others. However, ^{1}H NMR can be used as a qualitative method to show the influence of the concentration of the nucleophiles on the total reactions. If pyridine is diluted 16 times with DMSO, no nucleophilic substitution is observed (**3a**–**d**). In contrast to this, a temperature-dependent substitution of tosylcellulose with pure pyridine occurred (**3e**–**j**). In the case of butylamine, the reaction speed without using additional solvent is also faster than that in the presence of DMSO (see Table 1). This means that besides the solvent effect, the concentration of the nucleophile is also very important for the nucleophilic substitution.

A quantitative 2D H H COSY NMR spectrum is required in the case of overlapping peaks in the ^{13}C NMR spectra in partially substituted cellulose derivatives or at C-2 and C-3 positions where overlapping has been reported in the literature.[34] This is also the case if our target products would be further modified completely in the C-2 and C-3 positions.

The accuracy of the quantitative ^{13}C NMR results is very well known with the structurally more complex polysaccharide, heparin.[37–39] Recently, we reported the accuracy of the ^{13}C NMR results for cellulose derivatives calculated via the 'triangle method'.[20] We used additional three different independent analytical methods to support the quantitative ^{13}C NMR results ('triangle method'), namely elemental analysis, ESCA powder analysis and fluorescence-labelled reagents. The reaction with a fluorescence-labelled reagent showed the closest correlation with the results of ^{13}C NMR spectroscopy. There was only a one-percent deviation. But one has to take into account that an additional reaction step for characterizing the functional group by the fluorescence method is, for the most part, not complete; therefore one-percent deviation can be accepted. A good example is the Merrifield solid-phase synthesis of proteins, which require a long experimental period to reach 99% completion.[40] A two-percent deviation from the results of ^{13}C NMR was observed by elemental analysis, and the ESCA powder method showed a four-percent deviation.[20]

Previously, we could show that the tosyl groups in the C-2 and C-3 positions of cellulose were completely removed by several reduction steps at the end of the regioselective and complete C-6 substitution reaction pathway.[20] Because this reduction procedure was not new, we did not repeat the detosylation reaction with our new exclusively C-6 substituted derivatives.

It is clear that our target products cannot show any configurational change at the C-6 position; however, one could argue that the reductive detosylation in the C-2 and C-3 positions of cellulose or nucleophilic substitution could lead to a change of configuration. From numerous cellulose tosylation reactions reported in the literature, reductive detosylation was only shown with one derivative,[20] and there was no configuration change observed. This is further supported in that no configuration change was observed during the reductive detosylation of glucose.[41]

Apart from the above-mentioned few completely substituted cellulose products, mostly partially substituted cellulose derivatives are described in the literature.[25–33] This means that the distribution of the derivatized groups can be arranged along the polymer chain in a different way dependent on reaction conditions: in homogeneous solution or under heterogeneous conditions. The result could be either a Bernoulli, blockwise or other distribution along the polymer chain.[16,34] Only in a few cases, the identity of the distribution of these derivatized groups have been characterized.[16,42] This is very important for describing the purity and

identity of the substances. Partially substituted cellulose derivatives do need lots of additional analytical characterization methods for describing their purity. In our case, with complete substitution in the C-6 position of cellulose, the result is a defined homogeneous distribution of butylamino and pyridyl residues along the polymer chain. From this point of view, our target derivatives are automatically characterized along the polymer chain.

If we add up all of the above-mentioned arguments, the well-characterized 6-butylamino-6-deoxycellulose and 6-deoxy-6-pyridiniumcellulose derivatives are a milestone in the preparation of new defined derivatives with full substitution in the C-2 and C-3 positions of cellulose. The results in all cases would be a well-defined structure (homopolymer-like) along the polymer chain and not the usual reported partially substituted products, which are not fully characterized in the literature, with probably Bernoulli, block and other structures along the polymer chain.

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